Preface

Cancer is emerging as a leading cause of death in poorer countries where the majority of the World's population lives. Moving Cancer treatment of global health settings has been seen as costly. Challenging or even hopeless, so relatively little effort has been devoted to this problem. However, early detection may lead to more affordable and effective cancer treatment so new diagnostics technologies have the potential to help overcome global healthcare disparities for cancer.

One of the major areas in cancer research is targeted delivery of drugs to cancerous cells, which can not only increase the therapeutic efficacy but also reduce the adverse side effects of the drugs.

Most current technologies for cancer detection and diagnostics are not suitable for low income, low resource countries, but there is no concerted and coordinated effort to developing more appropriate technologies for widespread global health use. This book represents an organized effort to discuss new, low-cost technologies with potential for cancer detection and treatment in low resource countries.

The goals of this book are to bring together cancer clinicians and researchers discuss the state of the science, focusing on clinical aspects of cancer and on promising technological developments for diagnosis and treatment.

Thank You,
Dr. Ahmed M Malki
About Editor

Dr. Ahmed M Malki is currently Associate professor and Director of Biomedical Program, Health Sciences Department, College of Arts and Sciences, Qatar University, Doha. He graduated from Edison Institute of Biotechnology at Ohio University (USA) in 2006 with PhD in Molecular Oncology then He joined Nutritional Science and Toxicology department at University of California Berkeley, USA in 2007. Then, he was appointed as director of Genetic Engineering laboratories at center of excellence at City of Research and Technology. He is an editorial board member in several prestigious journals in biochemistry and cancer research. He received best research record award from Ohio University in 2006, best research paper from global breast cancer conference in Seoul, South Korea in 2011 and best research award from Annual Research Day 2014 in Qatar. He also received Alexandria University award for scientific encouragement and achievement in 2011. He is currently a leader of group of no of grants in the field of molecular therapeutics. The central theme of Dr. Malki’s research is the understanding of the signaling pathways that regulate cell proliferation and cell death and designing novel anticancer drugs. Signaling transduction pathways and networks have recently proven to be attractive targets for cancer therapy. Dr. Malki’s laboratory is also interested in designing novel anticancer drugs that could be used alone or could be used as a therapeutic in conjunction with radiation for the treatment of cancer as radio sensitizers. More specifically, EGFR-Ras-PI3K-PTEN-Akt pathway which is the major radio protective pathway active in most solid tumors and this pathway then presents targets that could be manipulated in a clinical setting to modify the radiation response. Dr. Malki’s laboratory is interested in developing small molecules that specifically trigger cancer cells to undergo apoptosis and act as radiosenitizers. He is currently a leader of group of no of grants in the field of molecular therapeutics.
Acknowledgement

I would like to express my gratitude to the many people who saw me through this book; to all those who provided support, talked things over, read, wrote, offered comments, allowed me to quote their remarks and assisted in the editing, proofreading and design.

I would like to thank (OMICS International Group) for enabling me to publish this book. Above all I want to thank my wife and the rest of my family, who supported and encouraged me in spite of all the time it took me away from them. It was a long and difficult journey for them.

Grateful and deepest thanks are also extended to my family, friends and students for their caring and support. They have been wonderful supporter and I would not be here today if it were not of them.

Last and not least: I beg forgiveness of all those who have been with me over the course of the years and whose names I have failed to mention.
Introduction

Cancer has been known since human societies first recorded their activities. It was well known to the ancient Egyptians and to succeeding civilizations but, as most cancers develops in the latter decades of life, until the expectation of life began to increase from the middle of the nineteenth century onwards, the number of people surviving to this age was relatively small. Now that the infectious diseases, the major causes of death in the past, have been controlled by improvements in public health and medical care, the proportion of the population at risk of cancer has increased dramatically. Although diseases of the heart and blood vessels are still the main cause of death in our ageing population, cancer is now a major problem. At least one in three will develop cancer and one in four men and one in five women will die from it. For this reason, cancer prevention and control are major health issues. However, cancer research has wider significance.

Cancer is not confined to man and the higher mammals but affects almost all multicellular organisms, plants as well as animals. Since it involves disturbances in cell proliferation, differentiation, and development, knowledge of the processes underlying this disease help us to understand the very basic mechanisms of life.

After a general introduction describing the pathology and natural history of the disease, each section gives a more detailed, but nevertheless general, survey of its particular area. We have tried to present principles rather than a mass of information, but inevitably some chapters are more detailed than others. Each chapter gives a short list of recommended reading which provides a source for seekers of further knowledge. The topics covered have been selected with some care. Although some, particularly those concerned with treatment, may not at first glance appear to be directly related to cell and molecular biology, we feel that knowledge of the methods used must give a wider understanding of the practical problems which may ultimately prove to be solvable by the application of modern scientific technology. On the other hand, knowledge of inherent cell behavior (e.g. radiosensitivity, cell cycling, development of drug resistance, etc.) is important for the design of novel therapeutic approaches that rely less on empirical considerations. Despite differences in the levels of technical details presented in some chapters, we hope that all are comprehensible. Finally, the editors would appreciate any comments, suggestions or corrections should a second edition prove desirable.
<table>
<thead>
<tr>
<th>Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chapter-1</strong>: Theory of cancer and cancer progression</td>
<td>1-24</td>
</tr>
<tr>
<td><strong>Chapter-2</strong>: Endocrine Tumors</td>
<td>25-40</td>
</tr>
<tr>
<td><strong>Chapter-3</strong>: Tumor Angiogenesis and Anti- Antigenic Therapy</td>
<td>41-54</td>
</tr>
<tr>
<td><strong>Chapter-4</strong>: Mutation prevention</td>
<td>55-68</td>
</tr>
<tr>
<td><strong>Chapter-5</strong>: Tumor Immunology and Immunotherapy</td>
<td>69-82</td>
</tr>
<tr>
<td><strong>Chapter-6</strong>: Cancer Targeting Strategies</td>
<td>83-98</td>
</tr>
<tr>
<td><strong>Chapter-7</strong>: Recent Advances in Molecularly Targeted Therapies for</td>
<td>99-125</td>
</tr>
<tr>
<td>Endometrial Cancer and their Future Clinical Implications</td>
<td></td>
</tr>
<tr>
<td><strong>Chapter-8</strong>: Herbal Treatment Strategies for Breast Cancer</td>
<td>126-141</td>
</tr>
<tr>
<td><strong>Chapter-9</strong>: Anticancer Diet</td>
<td>142-151</td>
</tr>
<tr>
<td><strong>Chapter-10</strong>: Surgical Strategies of Cancer Treatment with Special</td>
<td>152-167</td>
</tr>
<tr>
<td>Reference to Differentiated Thyroid Cancer</td>
<td></td>
</tr>
<tr>
<td><strong>Chapter-11</strong>: Surgical Strategies in the Treatment of Parathyroid</td>
<td>168-185</td>
</tr>
<tr>
<td>Diseases</td>
<td></td>
</tr>
</tbody>
</table>
Abstract

This chapter on tumor genesis begins with a description of the normal structure of cells and tissues, followed by a discussion of normal process of growth of both cells and tissues. It follows the definition of the cancer and the process of carcinogenesis and finally the abnormal process leading to carcinogenesis such as the early mutational events in carcinogenesis, microRNAs in human cancer and cancer stem cell hypothesis and microenvironment.

Introduction

Cancer has been known since human societies first recorded their activities. It was well known to the ancient Egyptians and to succeeding civilizations but, as most cancers develops in the later decades of life, until the expectation of life began to increase from the middle of the nineteenth century onwards, the number of people surviving to this age was relatively small. Now that the infectious diseases, the major causes of death in the past, have been controlled by improvements in public health and medical care, the proportion of the population at risk of cancer has increased dramatically. Although diseases of the heart and blood vessels are still the main cause of death in our ageing population, cancer is now a major problem. At least one in three will develop cancer and one in four men and one in five women will die from it. For this reason, cancer prevention and control are major health issues. However, cancer research has wider significance. Cancer is not confined to man and the higher mammals but affects almost all multicellular organisms, plants as well as animals. Since it involves disturbances in cell proliferation, differentiation, and development, knowledge of the processes underlying this disease help us to understand the very basic mechanisms of life. About 140 years ago a German microscopist, Johannes Mueller, showed that cancers were made up of cells, a discovery which began the search for changes which would help to pinpoint the specific differences between normal and cancer cells. In the intervening period a huge amount of information has been acquired about the cancer cell. In the past two decades in particular, rapid technological progress has allowed us to begin to dissect the cancer genome, transcriptome, and proteome in unprecedented detail and today there seems no limit to the amount of information that can be obtained. However,
this does not naturally answer all of the questions posed by those early cancer biologists. Some fundamental questions remain unanswered, despite our technical prowess and the availability of commercial ‘kits’ for most basic assays. Even the most advanced technology is of no value if it is not applied appropriately and it is still too early for the benefits of some recent technical advances to be clear. In the past, some of the major questions for the cancer biologist concerned what types of experiments were possible and the development of new techniques to extend these possibilities formed a major part of the work done. Now that almost anything seems technically possible, the key issue for the twenty-first century biologist is to identify the right questions to ask. This can make the difference between a deluge of uninterpretable data and a real improvement in understanding. This book does not aim to identify what these ‘right’ questions are but to provide an introduction to current understanding of cancer, its causes, biology, and treatment. However, we do indicate areas in which new and exciting discoveries are being made and those in which key questions remain unanswered.

Cancer is a disorder of cells and although it usually appears as a tumour (a swelling) made up of a mass of cells, the visible tumour is the end result of a whole series of changes which may have taken many years to develop. In this chapter, we discuss in general terms what is known about the changes that take place during the process of tumour development, consider tumour diagnosis and nomenclature, and provide some definitions. Succeeding chapters deal with specific aspects in more detail.

**Normal Cells and Tissues**

The tissues of the body can be divided into four main groups: the general supporting tissues collectively known as mesenchyme, the tissue-specific cells-epithelium, the ‘defence’ cells-the haematolymphoid system and the nervous system. The mesenchyme consists of connective tissue- fibroblasts which make collagen fibres and associated proteins, bone, cartilage, muscle, blood vessels, and lymphatics. The epithelial cells are the specific, specialized cells of the different organs, for example, skin, intestine, liver, glands, etc. The haemato-lymphoid system consists of a wide group of cells, mostly derived from precursor cells in the bone marrow which give rise to all the red and white blood cells. In addition, some of these cells (lymphocytes and macrophages) are distributed throughout the body either as free cells or as fixed constituents of other organs, for example, in the liver, or as separate organs such as the spleen and lymph nodes. Lymph nodes are specialized nodules of lymphoid cells, which are distributed throughout the body and act as filters to remove cells, bacteria, and other foreign matter. The nervous system is made up of the central nervous system (the brain and spinal cord and their coverings) and the peripheral nervous system, which is comprised of nerves leading from these central structures. Thus, each tissue has its own specific cells, usually several different types, which maintain the structure and function of the individual tissue. Bone, for example, has one group of cells responsible for bone formation and a second group responsible for bone resorption and remodeling when the need arises, as in the repair of fractures. The intestinal tract has many different epithelial cell types responsible for the different functions of the bowel, and so on. The specific cells are grouped in organs which have a standard pattern (Figure 1). There is a layer of epithelium, the tissue-specific cells, separated from the supporting mesenchyme by a semipermeable basement membrane. The supporting tissues (or stroma) are made up of connective tissue (collagen fibers) and fibroblasts (which make collagen), which may be supported on a layer of muscle and/or bone depending on the organ. Blood vessels, lymphatic vessels, and nerves pass through the connective tissue and provide nutrients and nervous control among other things for the specific tissue cells. In some instances, for example, the skin and intestinal tract, the epithelium which may be one or more cells thick
depending on the tissue, covers surfaces. In others it may form a system of tubes (e.g. in the lung or kidney), or solid cords (e.g. liver), but the basic pattern remains the same. Different organs differ in structure only in the nature of the specific cells and the arrangement and distribution of the supporting mesenchyme.

![Figure 1: A typical tissue showing epithelial and mesenchymal components.](image)

### Control of Growth in Normal Tissues

The mechanism of control of cell growth and proliferation is one of the most intensively studied areas in biology. It is important to make the distinction between the terms ‘growth’ and ‘proliferation’. Growth is used here to refer to an increase in size of a cell, organ, tissue, or tumor and proliferation to an increase in the number of cells by division. ‘Growth’ is often used as a loose term for both of these processes but the distinction is particularly important now that factors controlling both of these processes are becoming clear. In normal development and growth there is a very precise mechanism that allows individual organs to reach a fixed size, which for all practical purposes, is never exceeded. If a tissue is injured, the surviving cells in most organs begin to divide to replace the damaged cells. When this has been completed, the process stops, that is, the normal control mechanisms persist throughout life. Although most cells in the embryo can proliferate, not all adult cells retain this ability. In most organs there are special reserves or stem cells, which are capable of dividing in response to a stimulus such as an injury to replace organ-specific cells. The more highly differentiated a cell is, for example, muscle or nerve cells, the more likely it is to have lost its capacity to divide. In some organs, particularly the brain, the most highly differentiated cells, the nerve cells, can only proliferate in the embryo, although the special supporting cells in the brain continue to be able to proliferate. A consequence of this, as we shall see later, is that tumors of nerve cells are only found in the very young and tumors of the brain in adults are derived from the supporting cells. In other tissues there is a rapid turnover of cells, particularly in the small intestine and the blood and immune system. A great deal of work has been done on the control of stem cell growth in the red and white cells (haemopoietic system), and the relationship of the factors involved in this process to tumor development. For reasons that are still unclear, rapid cell division itself is not necessarily associated with an increased risk of tumor development, for example, tumors of the small intestine are very rare. In the embryo there is a range of stem cells, some cells capable of reproducing almost any type of cell and others with a limited potential for producing more specific cells, for example, liver or kidney. In the adult, there is now unequivocal evidence for the existence of stem cells capable of perpetuating themselves through self-renewal to generate specialized cells of particular tissues. Striking parallels exist between the properties of stem cells and cancer cells. This, together with the potential for the use of human stem
cells in various types of regenerative medicine, makes this a very active area of research [1]. Control of organ or tissue size is achieved via a fine balance between stimulatory and inhibitory stimuli. When the balance is shifted, for example, when the tissue is damaged and repair is needed, when a specific physiological stimulus is applied, for example, hormonal stimulation or because extra work is required from an organ, the component cells may respond in one of two ways to achieve these objectives. This may be by hypertrophy, that is, an increase in size of individual components, usually of cells which do not normally divide. An example is the increase in size of particular muscles in athletes. The alternative is hyperplasia, that is, an increase in number of the cells. When the stimulus is removed, commonly the situation returns to the status quo as exemplified by the rapid loss of muscle mass in the lapsed athlete. Some of the stimuli that lead to these compensatory responses are well-known growth factors and hormones that are discussed in more detail in Chapter 5. Recent work on the insulin/IGF (insulin-like growth factor) system, particularly in the fruit fly Drosophila, has demonstrated that this plays a pivotal role in the control of organ and organism size [2]. It is of note that several molecules involved in these processes are known to act as oncogenes or to be deregulated in cancer. For example, IGFs are commonly overexpressed and the Phosphoinositide3-Kinase (PI3K) pathway, which is activated by insulin/IGF signaling, is functionally disrupted in various ways in cancer cells [3].

The Definition of Cancer

As humans we are comprised of many millions of cells. Some cells are specific to certain tissues, for example epithelial cells are found throughout the gastrointestinal tract, bladder, lungs, vagina, breast and skin. This group of cells accounts for approximately 70% of cancers [4,5]. However, any cell has the potential to undergo malignant changes and lead to the development of a carcinoma. Cancerous cells are not confined to localized ‘overgrowth’ and infiltration of surrounding tissue, but can spread to other parts of the body via the lymphatic system and bloodstream, creating secondary deposits known as ‘metastases’ [6-8]. This can occur when ‘normal’ cell control mechanisms become disrupted or indeed fail [5]. Surgical removal of the original tumor is not always a successful treatment in malignant disease, due to microscopic spread. Malignant tumors are often irregular in shape, with ill-defined margins [7,9]. The potential for microscopic spread occurs when the tissue surrounding the visible tumor appears to the eye (macroscopic examination) to be unaffected by cancer. Microscopic examination of the surgical resection margins can reveal the presence of malignant cells. If left untreated, these cells will result in localized recurrence of the cancer and eventual spread (metastasis). The spread of the malignant cells extends outward from the original tumor, and has been described as resembling the appearance of a crab. This is the origin of the term ‘cancer’, which was derived from the Latin meaning ‘crab’ [7]. The earlier a cancer is detected, the less likely it is to metastasize, and so the more favorable the prognosis for the individual [10].

Metastatic spread

All cells replicate themselves. This usually happens about 50-60 times before the cell eventually dies (see Chapter 4) [5,11]. However, as malignant cells replicate, they grow in an irregular pattern, infiltrating surrounding tissue. This can result in infiltration of the lymphatics and/or blood vessels. By gaining access to these vessels, malignant cells can be carried to other sites within the patient’s body, where they will replicate and grow, rather like rodents establishing colonies in various parts of a town by gaining access to sewer systems [7,9]. In order to ensure that these malignant cells receive the nourishment they need to thrive, angiogenesis occurs. This is the formation of new blood vessels [11].
Lymphatic Spread: Malignant cells gain access to the lymphatic system and travel along the vessels to the ‘regional draining’ lymph nodes [7]. The malignant cells can then establish residency in these regional nodes, where they replicate and eventually replace the lymph node with a malignant tumour - that is, cancer. Malignant cells from this tumour can then travel, via the lymphatic system, to the next group of lymph nodes, thereby spreading the malignancy throughout the patient’s body [7]. Lymphomas and squamous cell carcinoma of the head and neck are two examples of where cancer commonly spreads via the lymphatic system [11].

Blood Spread: As with lymphatic spread, malignant cells can also infiltrate the vascular system and travel along the vessels until they arrive at an area where they can become lodged and subsequently replicate to form a secondary (metastatic) deposit. The malignant cells can then migrate via the smaller blood vessels - that is, the capillaries [7]. However, there is evidence that only a small percentage of cells entering the vascular system actually survive to give rise to blood-borne metastatic spread [7]. Malignancies which are linked to blood-borne spread include melanoma and small cell carcinoma of the lung [11].

Liver: The commonest site for blood-borne metastases is the liver. Malignancies originating from the gastro intestine, including the pancreas, commonly metastasize to the liver. Other malignancies which can result in secondary deposits in this organ include breast, melanoma, lung and urological cancers [7,9].

Lung: The lung is the second most common site for metastatic spread. Tumors that are associated with metastasizing here include the breast, teratomas, melanomas and sarcomas [7,9].

Bone: Bone metastases are commonly associated with malignancies of the breast, prostate, kidney, lung and thyroid. Patients with bone metastases can often present with pain. Pathological fractures are not uncommon due to the damage caused to the bone by the malignant cells - that is, the cancer cells replacing the healthy cells and thereby weakening the bone, making it more prone to fracture [7,9].

Brain: Brain metastases are closely associated with primary malignancies of the lung, but can also arise from other sites, including the breast, teratomas and malignant melanoma [7,9].

Adrenal glands: Breast and lung primary malignancies are more frequently associated with secondary deposits in the adrenal glands, compared to cancers arising from other sites within the body [7,9].

Transcoelomic spread: Transcoelomic spread is the term used to describe invasion of the serosal lining of an organ by malignant cells. The malignant cells trigger an inflammatory response, which results in a serous exudate. This is commonly seen in the peritoneal cavity, where it is associated with ovarian and colonic malignancies [7,9].

The Process of Carcinogenesis

Carcinogenesis (the process of cancer development) is a multistage process (Figure 2). In an animal, the application of a cancer-producing agent (carcinogen) does not lead to the immediate production of a tumour. Cancers arise after a long latent period and multiple carcinogen treatments are more effective than a single application. Experiments carried out on mouse skin in the 1940s by Berenblum and Shubik [12] indicated that at least three major stages are involved. The first was termed initiation and was found to involve mutagenic
effects of the carcinogen on skin stem cells. The second stage, which can be induced by a variety of agents that are not directly carcinogenic in their own right, was termed promotion. Following chronic treatment of carcinogen-initiated mouse skin with promoting agents, papillomas (benign skin tumours) arise. The major effect of promoters seems to be their ability to promote clonal expansion of initiated cells. Finally in the third stage, progression, some of these benign tumours either spontaneously or following additional treatment with carcinogens, progress to invasive tumours. The terms coined to describe this animal model are still commonly applied to describe the process of carcinogenesis in man. The mouse skin model indicated that carcinogenesis is a multistep process and clearly this is also the case for human cancer. For example, most solid tumors of adults arise in the later decades of life, usually a long time after exposure to a specific carcinogenic insult or after a long period of continuous exposure and this can be explained in terms of the requirement for several distinct heritable changes. The nature of some of these changes is now known in detail and is discussed at length in several of the following chapters. These include genetic alterations to proto-oncogenes and tumor suppressor genes (Chapter 2) and epigenetic alterations.

Histopathological observations also provide evidence for a long preneoplastic period, sometimes with morphologically identifiable lesions such as benign tumors or in situ dysplasia, which may persist for many years and within which a malignant tumor eventually arises. The latent period between initiation and the appearance of tumors is great. In man, after exposure to industrial carcinogens, it may take over 20 years before tumors develop. Even in animals given massive doses of carcinogens, it may take up to a quarter or more of the total lifespan before tumors appear. The requirement for acquisition of multiple events is the likely explanation for this. In the tumor that finally emerges, most of the genetic and epigenetic changes seen are clonal, that is they are present in the entire population of cells. It is likely that a series of selective phases of clonal expansion takes place in the tumor such that after each event, there is outgrowth of a clone of cells with a selective advantage. Evidence for this has come from studies on many tissues and particularly where areas of surrounding tissue or multiple related lesions can be sampled at surgery. In these circumstances, it is common to find several shared clonal events in different lesions and occasionally in the apparently ‘normal’ surrounding epithelium and additional events in the most histopathologically advanced lesion.

![Figure 2: Tumor development showing progression from normal to invasive tumor via accumulation of heritable changes over a long period of time. The rate of acquisition of these changes will be influenced by environmental exposures and host response.](image)
Early Mutational Events in Carcinogenesis

Alterations of the genetic code

Analysis of the DNA of tumor cells reveals that a finite number of gene mutations are responsible for the transmission of the phenotypic changes characteristic of the tumor from one cell to the other during cell division. These mutations may have arisen sporadically in a somatic cell through mis repair of endogenous DNA damage arising from oxidative stress and DNA replication errors, or through mistakes in somatic recombination events. Alternatively, they may be induced exogenously through the DNA-damaging action of environmental agents, such as ionizing radiation, UV and mutagenic alkylating or intercalating agents. Failure of the damage control processes to correct the damage before it is incorporated permanently into the genome during replication is critical. Phenotype or may even leave the encoded amino acid unchanged (silent mutations). Occasionally, the single base change may generate a premature stop codon, truncating the protein, which frequently leads to rapid degradation of the abnormal protein by the misfolded protein recognition system in the endoplasmic reticulum and the proteasome. Insertions and deletions of a single base alter the reading frame of the gene. As most genes have evolved with multiple stop codons protecting the two non-coding frames, the frame-shifted sequence will most probably contain a stop codon close to the position of the insertion/deletion. In some infrequent instances, the mutated single base may lie in a critical structural element of the gene, such as the promoter site regulating gene activity, or in a recognition site critical for RNA processing, for example splice site mutations resulting in exon skipping deletions in the E-cadherin gene [13]. In addition to the intragenic mutations described above, there is a range of additional mechanisms whereby the genome may become perturbed during tumor development. Alterations in the copy number of cellular genes are commonly described in human tumors. Both allelic gains and losses are encountered, and their biological consequences are described elsewhere in this review. Amplification of genetic regions may take the form of intrachromosomal duplications, leading to the in situ amplification of a gene with oncogenic potential. Transcription of the amplified gene complex subsequently leads to overexpression of the gene product. Alternatively, the amplification may occur extra chromosomally, leading to the formation of multiple copies of chromosomal fragments (double minutes) containing one or more transcriptionally active genes with an oncogenic capacity. The spectrum of mutational events in tumor cells can also include chromosomal translocation and inversion events leading to the structural rearrangement of parts of the genome. This may result in a fusion of two unrelated gene fragments, creating a chimeric gene instructing production of a protein with abnormal function. Alternatively, the rearrangement may transpose an endogenously active promoter to coding sequences from a gene that is normally either tightly regulated or transcriptionally silent in the tissue. This form of mutation leads to the inappropriate expression of the protein, for example, in parathyroid tissue where the CCND1 (Cyclin D1) gene is placed under the control of the highly active parathyroid hormone gene promoter [14]. This is also seen in thyroid tissue where the transcriptionally inactive glial-derived Neurotrophic Factor Receptor (RET) tyrosine kinase gene is placed under the control of one of a number of different promoters active in thyroid tissue [15]. As a result of this translocation event, the neuroendocrine tissue-restricted RET protein is produced in thyroid cells and delivers cell proliferation signals in a ligand-independent. Functional translocations are also frequent in the lymphoid and myeloid lineages, presumably due to the propensity of these cells to undergo chromosomal rearrangements during immunoglobulin and T cell receptor maturation. Failure to restrict the high level of chromosomal rearrangement activity to the correct locus may explain the abundance of such alterations in immature stages of the lineages. In solid
tumors translocations are seen primarily in the endocrine tissues mentioned above and in the paediatric tumors rhabdomyosarcoma and Ewing’s sarcoma, both of which involve activation of genes regulating developmental pathways. Translocations are reported less frequently in other solid tumors, and here their biological relevance remains uncertain. Significantly, in none of the solid tumor types showing translocations is there any evidence for endogenous chromosomal rearrangement processes that could explain the phenomena. Two non-mutational events are also implicated in the changes in gene expression during ontogenesis. In the first situation, transcriptional silencing of an essential tumor suppressor gene is associated with non-mutational changes to the structure of the gene promoter region. Changes in the methylation status of individual nucleotides of the DNA, as well as to the methylation and acetylation status of the DNA-binding histone core proteins, are involved in regulating local gene expression. A second non-mutational event is discussed below, where gene silencing through endogenous RNA binding microRNA molecules has been suggested to be an additional step in transcriptional control, leading to silencing in a post-transcriptional manner. An altogether different mutational mechanism is seen almost exclusively in animal model systems, where insertion of retroviral sequences or retroviral-like elements into the genome results in the disruption of cellular genes. In humans, the role of insertional mutagenesis is less clear. Retroviral insertion leading to proto-oncogene overexpression has been implicated in the development of retroviral gene therapy-associated lymphoproliferative malignancies in a small number of cases. Nevertheless, the general applicability of this mutational mechanism for human cancer is unclear, and it is certainly uncommon. In addition to retroviral insertion, viruses have evolved a range of strategies for productive infection of mammalian cells that subvert defense and regulatory pathways. As a consequence of these actions, the viral proteins elicit an oncogenic action through growth stimulation, suppression of apoptosis or inactivation of endogenous tumor suppressor gene function.

**Events Accompanying Progression**

Mathematical and molecular studies on tumor tissues have each established that tumors can arise and develop through a series of intermediate stages. The clonal expansion paradigm suggests that discrete stages arise through evolutionary selection of appropriate phenotypes that are themselves defined by mutational events. Histopathologic studies deliver a partially convergent concept, where morphologically distinct stages of tumor formation and development are discernable in almost all tumor entities. The combination of the morphological models of tumor development and analysis of molecular events suggests that tumor development indeed follows a series of steps from pre-cancerous lesions (hyperplasia, atypical hyperplasia) that lead either directly or indirectly to full neoplasia (infiltrative and metastatic growth). During this progression, the normally differentiated phenotype may become either partially or completely lost [16]. Estimates of the number of mutations and steps that are required to create a full malignant phenotype vary wildly. In vitro studies suggest that mutation of as few as three key genes is sufficient, whilst massive DNA resequencing studies of tumor cell genomes have revealed hitherto undiscovered complexity in the magnitude and diversity of DNA alterations; however, it remains unclear which of these, if any, are required for the acquisition of a malignant phenotype [17]. Three conceptual models can help in partly reconciling these differences. Kinzler and Vogelstein suggested, at least for the model of colon carcinogenesis, that there is a linear evolution of the cells within the developing tumor, which follows a well-circumscribed and sequential series of events [18,19]. Each step in their model is represented by the mutation of a single key gene. However, the analysis of the gene alterations present in different areas of some tumors shows that some clones lack the full complement of gene mutations. This may indicate that
a simple linear monoclonal evolution is not always followed [20]. An alternate view to the Vogelstein model is that mutations are acquired in a cumulative manner, with some clones in the tumor acquiring mutations that lead to them branching off to an evolutionary dead end and others only being required at specific points in the tumor development. Hanahan and Weinberg [21] have suggested that key cellular pathways related to functional changes in tumor cell biology are individually targeted by mutational events, explaining how the development of malignancy can result from a finite number of mutations. Finally, systems theory and pathway analysis suggest that each functional activity of the cell described by Hanahan and Weinberg requires multiple hits to remove backup and alternative pathways. It is, however, worthy of note that tumor cells cannot tolerate wholesale genomic alterations; consequently, there cannot be an unlimited number of mutations as some functional pathways are essential for continued cell survival. A discrepancy of orders of magnitude between the sporadic rate of mutational activity observed in cells and the level of mutations found in tumors has prompted Loeb [22] to suggest that a key process in tumor cell development must be the acquisition of a mutational activity (mutator phenotype, loss of caretaker function). Although tumor suppressor and apoptosis genes could be considered candidate mutator genes, no convincing evidence for a specific increase in mutation rate due to loss of these genes has been presented. Genes involved in maintaining genomic integrity, such as the DNA mismatch repair genes, whilst implicated in cancer susceptibility, provide no clear evidence of mutator-gene driven genome changes.

**Proliferation Modifying Genes**

A major category of the genes influencing cell proliferation contains members of signaling pathways involved in the regulation of cellular growth. At the cell surface this can be seen by the uncontrolled production of stimulatory growth factors, the abnormal expression of growth factor receptors or the production of a mutated form of the receptor that has acquired the capacity to autonomously engage and activate the downstream intracellular signaling cascade. A related functional set of tumor genes is that involved in the transmission of the growth-regulating signal to the transcriptional apparatus, which includes signal-transducing kinases and transcription factors. An additional group of proliferation genes plays a role in steering the transit of cells into, through and out of the cell cycle. Inappropriate functioning of these genes leads to uncontrolled cell cycle activity and the failure of proliferating cells to differentiate. In the case of cell cycle checkpoint control genes, this can allow cells with non-repaired DNA damage or chromosomal aberrations to continue through the cycle, yielding genetically aberrant daughter cells. Failure to eliminate damaged cells is an additional feature of the mutations influencing a further set of cancer genes, those involving the cellular pathways regulating programmed cell death (apoptosis and anoikis, a form of apoptosis that is induced in anchorage-dependent cells detaching from the surrounding cells and/or matrix). The failure of tumor cells to initiate a normal apoptotic death response after stress and/or mutation of DNA, or to initiate apoptosis after loss of cell-cell and cell-matrix contact, can involve inactivation of the intrinsic (mitochondrial) pathway and extrinsic (ligand-receptor) apoptosis-inducing pathways. This can be brought about by inappropriate overexpression of anti-apoptotic proteins or by inactivation of pro-apoptotic proteins. More recently, the protective sequestration of cells bearing oncogenic gene mutations into a pathway of Oncogene-Induced Senescence (OIS) has been described. The regulation of this pathway is poorly understood, but escape from growth restrictions imposed by the activation of the senescence programme appears to be a critical step in oncogenesis and may involve overcoming cell cycle arrest by removing expression of the p16 cyclin-dependent kinase inhibitor. It remains to be seen which other protein activities regulate entry and exit from OIS and how mutations of these genes influence tumorigenesis.
Acquisition of the Invasive/Metastatic Phenotype

Although changes in proliferative regulation pathways are critically important, the acquisition of an invasive/metastatic phenotype is a major step in solid tumor formation. The necessary changes in gene expression may occur through mutation or through changes in more global programmes of cell regulation, such as the Epithelial to Mesenchymal Phenotypic Transition (EMT). Tumor invasion into surrounding tissues requires distinct phenotypic alterations. Loss of cell-specific adhesion allows tumor cells to detach from neighbouring cells and the underlying extracellular matrix. This may be accompanied by upregulation of an alternative programme of adhesion, allowing the tumor cell to adhere to anomalous cells or matrixes (e.g. a switch from epithelial specific E-cadherin to the mesenchymal-cell specific cadherin’s in breast cancer) [23]. At the same time as acquiring an abnormal adhesive profile, the tumor cells may also develop a programme allowing for the degradation of the surrounding matrix proteins. Here, overexpression of specific proteases may facilitate local destruction of matrix that allows the non-adherent tumor cell to exit the parental tissue and migrate [24]. Recent evidence suggests that the mobilization of tumor cells may be driven by local gradients of cell-specific and tissue-specific chemokine molecules. Changes in the expression pattern of surface chemokine receptors of tumor cells may permit them to respond to a different chemokine milieu and has been suggested to be partly responsible for homing of tumor cells to specific distant sites such as bone marrow [25]. Separation of the tumor cell from surrounding parental tissue would normally be expected to initiate the anoikis programme of apoptosis, but as described above, this pathway is inactivated as part of the loss of proliferative regulation. The final stage in malignant growth, the acquisition of the capacity to generate new blood vessels that infiltrate the tumor and oxygenate the expanding cell mass, angiogenesis, is discussed in other chapter of this book.

Inherited Susceptibility

Within a population there are a proportion of individuals who are predisposed to develop cancer, either as an apparently sporadic disease or in response to an environmental challenge, such as exposure to tobacco smoke or ionizing radiation. The abnormally high frequency of some tumor types within related members of large families provided evidence that cancer is, in some circumstances, a heritable disease. Genetic linkage studies of these families has revealed that a number of these cancer syndromes occur as simple Mendelian traits, usually with a highly penetrant dominant pattern of inheritance. Many hereditary cancer susceptibility genes, such as Breast Cancer 1 and 2 (BRCA1/2) and the group of DNA mismatch repair genes, have a known function in the DNA repair. Incomplete functioning of DNA repair appears to render somatic cells highly susceptible to carcinogenetic noxae and spontaneous DNA mutations, leading to an accumulation of genetic damage and ultimately transformation. Other susceptibility genes involving impaired DNA repair lead to cancer prone syndromes such as xeroderma pigments, Bloom’s disease and Hereditary Nonpolyposis Colorectal Cancer (HNPCC), also known as Lynch syndrome. Yet, there are inherited susceptibility genes having no direct function in DNA repair, but still showing an autosomal dominant familial pattern. Von-Hippel-Lindau syndrome is a dominantly inherited hereditary cancer syndrome predisposing to a variety of malignant and benign tumors of the eye, brain, spinal cord, kidney, pancreas and adrenal glands. Other inherited cancer syndromes include ataxia telangiectasia, Li-Fraumeni syndrome, retinoblastoma, Wilms’ tumor, familial adenomatous polyposis, multiple endocrine neoplasias 1 and 2, just to mention a few. The hereditary mutations associated with cancer syndromes only have a big impact on the risk of a population if they are common. Thus, whilst mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 are found in almost 10% of women with breast cancer, the PTCH1 gene mutation responsible for the Gorlin/basal nevus syndrome
occurs in less than 1 per 50,000 of the population. However, it must be appreciated that the gene mutation frequencies vary considerably between populations, especially if the populations are isolated for geographical, religious or other reasons. Good examples in this context are BRCA2 mutations in Iceland and BRCA1/2 mutations among the Ashkenazi Jewish population. Inaccuracies in population estimates may bias clinical judgement and allocation of diagnostic resources [26]. Susceptibility to many diseases has been shown to be polygenic, with a multitude of low-penetrance common polymorphisms contributing to the risk of developing disease. These complex trait genes may contribute significantly to risk estimations of certain cancers. Therefore, it is useful to quantify the relative importance of known genes in the burden of disease by using the Population Attributable Fraction (PAF) that states the contribution of the studied gene to disease aetiology, independent of the environmental or other genetic factors that may interact with the gene in question [27]. New approaches, such as Genome-Wide Association Studies (GWAS) using Single Nucleotide Polymorphism (SNP) arrays, have provided tools to map and potentially identify some of the low penetrance hereditary cancer-susceptibility genes. Future developments here will require large-scale multinational collaborations, similar to those conducted on breast cancer [28].

**Genetic Instability, Clonal Selection, and Tumor Evolution**

Our recent ability to dissect the cancer genome at both the gross chromosomal and nucleotide level has revealed extensive genetic change. Often this is complex, particularly in advanced epithelial cancers and is commonly referred to as genetic or genomic instability. Recent studies have revealed that genetic instability can take distinct forms and a debate has arisen over whether these represent cause or effect. One type of instability is that which results from inactivation of Mismatch Repair (MMR) genes such as MSH2 and MLH1. Defects in MMR lead to numerous changes in short simple sequence repeats spread throughout the genome (called microsatellites; MMR is also termed Microsatellite Instability, MIN). MIN is characteristic of tumors found in patients with Hereditary Non-Polyposis Colorectal Cancer (HNPCC) who inherit mutations in MSH2 or MLH1. Interestingly, MIN tumors usually have a diploid karyotype which contrasts with non-MIN epithelial cancers which commonly show complex karyotype abnormalities, commonly termed Chromosomal Instability (CIN). The causes of CIN have been less obvious. There are several possibilities including alterations in mitotic checkpoint genes or genes involved in centrosome function or chromosomal segregation as discussed above. Already some tumors have been found to contain this type of alteration. It is also possible that once a cell has become aneuploidy by chance, this in itself predisposes it to become even more aneuploidy. This might happen, for example, at mitosis where segregation of aberrant or large numbers of chromosomes is more error-prone. A final mechanism is the inherent CIN which is generated in cells at senescence when chromosomes have severe telomere attrition. As indicated above, shortening of telomeres in advance of re-expression of telomerase can lead to severe chromosomal rearrangement via end-to-end fusion followed by breakage at segregation. There is evidence for all of these mechanisms and it is likely that one or more may contribute to the development of any given tumor and that the mechanism that is active will shape the genome in specific and recognizable ways that may well be tissue or tumor type specific. More detailed analysis of tissue samples taken throughout the course of tumor development should help to clarify these issues in the next few years.

While it is clear that tumors often have MIN or CIN, it is not yet clear whether this is an early event in the process, nor whether it is necessary for tumor development. It has been argued that the probability of tumors acquiring the necessary number of genetic alterations is too low without some additional mutator effect. This type of calculation is difficult and to
date no clear answer is apparent, though there is no doubt that some tumor cells have this phenotype while others, particularly early in their development, have little genomic alteration that can be identified. It is probable however, that the level of generation of mutations is critical and that too much instability is likely to impede tumor development rather than promote it. Already it is known that the type of genetic instability present in the tumor cell has an effect on the type of mutation found. Thus, for example, MIN colorectal tumors tend to inactivate the two alleles of the APC gene via two point mutations, whereas CIN tumors tend to have one point mutation and one allele lost by deletion. Two recent reviews explore these concepts in depth using what is known about colorectal carcinogenesis, possibly the best-studied model system, as an example [29,30].

**Selection of altered clones**

The process by which cancer cells develop and spread involves not only mutation but also selection of altered clones. These processes are the drivers of tumor evolution. It is thought that repeated rounds of mutation and selection occur during somatic evolution of a tumor. As the lineage evolves, the tumor cells acquire increased autonomy and eventually the capacity for metastasis. This is often compared to Darwinian evolution where in this case the fittest cell survives and multiplies. The low rate of mutation, calculated as $2 \times 10^{-7}$ per gene per cell division for cultured human cells [31] precludes the acquisition by a single cell of multiple mutations simultaneously. Even when large carcinogen doses are applied, the large number of potentially lethal mutations sustained at the same time as any set of mutations with potential advantage is likely to lead to cell death rather than instant tumorigenicity. Thus the expectation is that events occur singly and in a particular sequence in each cancer. This is frequently referred to as a genetic pathway or progression pathway and for several cancer types attempts have been made to map the pathway in genetic terms. As indicated above, colorectal cancer is arguably the best elucidated model in this regard [32]. In the colon, mutation of the APC gene is the initiating event. The resulting ‘early’ adenoma then commonly acquires mutations in KRAS, SMAD4, and TP53, respectively as it progresses histopathologically via ‘intermediate’ and ‘late’ adenoma to carcinoma. The frequency of each of these changes in each of the lesion types suggests that there is a preferred order of events in this case but this does not appear to be invariable. Results from other tumor types where samples can be obtained from lesions at different stages in the process, or from cancers with different malignant potential, also show shared lesions and temporal ordering of events in some cases. There is also evidence that alternative pathways can lead to the same result, and in different tissues specific mechanisms may dominate. For example, many tumors show inactivation of TP53 via mutation while some others show amplification of the negative regulator of p53, MDM2. In the Rb pathway, some tumors show direct mutation of RB while others show inactivation of the pathway via inactivation of the negative regulator p16. The order of events may differ in different tissues. For example, mutations of TP53 are found frequently in lung cancer but patients with a germline mutation in TP53 (Li–Fraumeni syndrome) do not develop lung cancer as part of the syndrome. Possibly this reflects inability of loss of p53 function to act early in the pathway to lung cancer but its suitability as an early event in the other tumours that develop in these Li–Fraumeni patients. The ultimate result of clonal evolution is escape from the normal growth restraints imposed on the cell in its normal tissue milieu. It follows therefore that the way in which this is achieved will depend to a great extent on what those growth restraints are. Hence the finding of tissue-specific and cell-specific genetic alterations, different timing of alterations etc., it is easy to envisage selection of mutations that increase proliferation or allow resistance to apoptosis or any of the other key features of
cancer cells. However, mutations that increase mutation frequency such as those that generate CIN or MIN do not in themselves confer an immediate advantage to the cell. At present, it is not clear how such a phenotype is selected. One plausible explanation is that such mutations may occur rarely in the same cell as a second mutation that does confer an immediate advantage and thus are selected as ‘passenger’ or ‘bystander’ events. Many forms of treatment, for example, radiation and chemotherapy may provide additional mutagenic and selective stimuli and may precipitate the emergence of more aggressive variants. An obvious example is the destruction of X-ray-sensitive cells by X-ray treatment. If the tumour also contains X-ray-resistant cells, the cancer cells which are left after treatment will be X-ray resistant. Although progression is usually towards greater malignancy, this is not invariably so. There are a number of cases, unfortunately small, in which rapidly growing tumors have ceased to grow or even disappeared completely. Although we do not yet have a full explanation for this, some studies indicate that this may be related to the development of anti-tumor immunity in the host. Thus, a series of changes occur in a cell as carcinogenesis proceeds. As the tumor progresses, more and more normal characteristics are lost and it is common to observe what has been described as dedifferentiation within the tumor tissue. This refers to the loss of normal structure and cellular functions characteristic of the tissue. Specialized products of the cell, for example, secretions or structural components may no longer be produced as the cell begins to take on new characteristics. The loss of normal differentiated features is referred to by a pathologist as anaplasia and the degree of such changes identified in tissue sections is used by the pathologist to ‘grade’ tumors. In general, less well-differentiated tumors have a poorer prognosis than those that retain the differentiated characteristics of the normal tissue. As a rule, there is an approximate correlation between tumor grade and growth rate. The most differentiated tumors (low grade, i.e. Grade I) tend to be more slow-growing and the most anaplastic (high grade, i.e. III or IV) the more rapidly growing. Human breast cancers are graded in this way and it has been shown that about 80% of patients with well differentiated Grade I breast cancers will be alive and well at five years (and often much longer) but only 20% of patients with Grade IV tumors will survive for this time. It is of course equally obvious from these figures that although 80% of patients with Grade I cancers survive, 20% with the same structural type of tumor do not. Tumor growth and progression is influenced by factors other than tumor structure, and these may range from the rate of mutation and type of mutation they contain to the reaction of the patient’s own defense mechanisms. In recent years much effort has been made to identify additional tests that can be carried out in the pathology laboratory at the time of tumor diagnosis to add both diagnostic and prognostic (predictive) information and the search for molecular markers (proteins or DNA changes) that can supplement the repertoire of morphological tests is intense. In fact, there are many examples of success in identifying such markers for use in tumor classification, prediction of prognosis or response to therapy, disease monitoring, and markers that can be used as therapeutic targets. These are described in several of the other chapters of this book. Possibly, the identification of such markers has been the earliest and most clinically applicable result of the intense effort of the past two decades to characterize human tumours at the molecular level. More successes will undoubtedly follow.

**Tumor clonally**

We have alluded to clonal evolution during tumor development but what of the origin of the tumor? Tumor clonality refers to the cellular origin of cancers. A monoclonal tumor develops from a single progenitor cell and a polyclonal tumor develops from multiple cells.
In many tissues, a solitary primary tumor is the norm and this may or may not recur or progress. In this circumstance the question of clonally concerns only this single tumor and its direct descendants. However, in some tissues the situation is more complex and when the structure of the organ is examined in detail widespread abnormal pathology may be found. In such a tissue multicentric tumors are sometimes found. Could there be many cells involved in the generation of a tumor or does each tumor arise from a single initiated cell? The appearance of multiple preneoplastic lesions in a tissue has been described as a ‘field change’. Figures 3 and 4 illustrate such a possible field effect. These tissues show a gradation from benign to malignant (as in Figure 2) but here the ‘progression’ is in space rather than time. This has been particularly described in tissues such as the bladder, colon, oesophagus, and oral mucosa where there is a large epithelial surface available for study and in which essentially all of the cells have received similar exposure to environmental agents. Here the question of clonally can be addressed to each individual tumor that arises, that is, tumor clonality, but of equal interest both to biologist and clinician, is the relationship between all the lesions in a single patient. This can be referred to as the clonality of the disease. With the advent of molecular genetic techniques there has been an explosion of information concerning the genetic relationship of such synchronous lesions.

There are several possibilities based on the clonality of each lesion and of the overall disease in the patient:

a. Each individual tumour consists of lineages derived from multiple normal parent cells. Such a tumor would be described as polyclonal. A tumor derived from a few parent cells would be termed oligoclonal.

b. Each tumor has a single parental cell of origin and multiple tumors in the same organ arise via seeding or direct spread of cells. Each is therefore a monoclonal tumor and this is monoclonal disease.

c. The disease is polyclonal, that is, more than one initiated cell progresses to generate multiple tumors each of which is derived from a single cell (monoclonal).

There is in fact evidence for all three situations, though the majority of human cancers are solitary tumors of monoclonal origin and there is ongoing debate over whether true polyclonal tumors do exist [33]. The methods most commonly used to assess tumor clonality are X-chromosome inactivation and Loss of Heterozygosity (LOH) analysis by microsatellite typing. During the course of embryonic development in females, genes on one of the X chromosomes are silenced by methylation of cytosine residues in the promoter. Such methylation is heritably maintained and prevents transcriptional activation within the promoter region. This process is random and in any tissue, 50% of cells have methylation of each copy of X. A monoclonal tumor will therefore have inactivation of any gene on only one of its X chromosomes and this can be detected at the molecular level. Polymorphisms in X-linked genes have been used to identify individual parental alleles and when assessed in combination with the use of methylation sensitive restriction endonucleases, which cut only non-methylated DNA, assays for allele specific methylation can be developed. Several X-chromosome loci have been used including Glycerophosphate Kinase (PGK), Hypoxanthine Phosphoribosyl Transferase (HPRT), and the androgen receptor gene (HUMARA) [34]. Such analyses are restricted to female tissues and to those women who are heterozygous at the locus of choice, namely those that have distinguishable maternal and paternal alleles. While there are some problems in interpretation of X-inactivation assays for clonality, these assays have the significant advantage that the feature studied is not itself part of the neoplastic process.
Figure 3: Section of the edge of a squamous cell carcinoma of skin, with normal skin (a) on the left and increasing dysplasia (b) and (c) leading into the main mass of the tumour (d) below right. Stained with haematoxylin and eosin (×50).

(a) Normal skin (compare with Figure 1.1) showing mesenchyme below covered by normal epithelium with basal cells, more differentiated superficial cells and on top, layers of keratin formed from the superficial cells (×360).
(b) Dysplastic skin. There is an increase in the number of basal cells, which are more irregular than in the normal and there is a disturbance in the formation of keratin, which is clumped into an irregular dark mass in the surface layer instead of the more regular sheets in (a), that is, differentiation is disturbed.
(c) Cell overgrowth. The cells themselves are abnormal: they vary in shape and size, the nuclei are much larger than normal and some are deeply stained. The usually distinct separation between epithelium and stroma is not seen, suggesting that invasion may be taking place. The cells are still recognizable as skin cells. This would be diagnosed as a moderately well-differentiated squamous carcinoma (×360).
(d) The centre of the tumour is made up of a mass of irregular spindle-shaped cells with no recognizable skin features. This would be diagnosed as an anaplastic (undifferentiated) carcinoma (×360).

Figure 4: Detail of the areas marked in Figure 3 at higher magnification.

(a) Normal skin (compare with Figure 1.1) showing mesenchyme below covered by normal epithelium with basal cells, more differentiated superficial cells and on top, layers of keratin formed from the superficial cells (×360).
(b) Dysplastic skin. There is an increase in the number of basal cells, which are more irregular than in the normal and there is a disturbance in the formation of keratin, which is clumped into an irregular dark mass in the surface layer instead of the more regular sheets in (a), that is, differentiation is disturbed.
(c) Cell overgrowth. The cells themselves are abnormal: they vary in shape and size, the nuclei are much larger than normal and some are deeply stained. The usually distinct separation between epithelium and stroma is not seen, suggesting that invasion may be taking place. The cells are still recognizable as skin cells. This would be diagnosed as a moderately well-differentiated squamous carcinoma (×360).
(d) The centre of the tumour is made up of a mass of irregular spindle-shaped cells with no recognizable skin features. This would be diagnosed as an anaplastic (undifferentiated) carcinoma (×360).

Microsatellite typing uses the Polymerase Chain Reaction (PCR) reaction to amplify short DNA products containing simple sequence repeat that are highly polymorphic in the human genome. Because the repeat length varies, alleles inherited from each parent are
frequently distinguishable by size. In a tumor with genomic deletion, for example, deletion of one copy of a tumor suppressor gene, loss of one allele (LOH, Loss of Heterozygosity) at nearby microsatellite repeats is commonly found in the tumor. Indeed this method has been widely used to map the locations of novel tumor suppressor genes. However, the use of this assay or any tumor specific molecular alteration to assess clonally per se is somewhat restricted. For example, LOH analysis cannot detect true cellular polyclonality since LOH in a mixed population is difficult to detect. When tumor-specific genetic markers are used for clonally analysis, it is predicted that in related monoclonal tumors, markers associated with changes that occurred early in tumor development will show greatest concordance and those that occur later in tumor development may show divergence in related tumors that have undergone clonal evolution. This latter observation can be referred to as sub-clonal evolution. LOH analysis has been used to assess the temporal sequence of genetic events that has taken place during the evolution of a tumor from a series of temporally or spatially related lesions. Examples of such studies include the elucidation of neoplastic lineages in Barrett oesophagus [35] and in synchronous and metachronous bladder cancer [36]. An example of the type of lineage deduced from such studies is shown in (Figure 5). Not only does information about the relationships between such lesions give valuable biological information about disease development but it also provides information with potential clinical application. For example, the presence of multiple different tumor clones within an epithelium may show a relationship to tumor recurrence rate or time to recurrence and may indicate a need for more vigilant monitoring. In the future, as targeted therapies become available, knowledge of the specific molecular characteristics of all tumors present may influence choice of therapy and polyclonal tumors may be more difficult to target in this way. By the time a tumor is detectable clinically, whether it has arisen from one or many cells, it has been present for a long time and the cells have had to go through a large number of cell divisions. A tumor of about 0.5 cm in diameter, which is just detectable, may contain over 500 million cells. Within such a population, even if deemed monoclonal by X-inactivation and other genetic assays, it is likely that at any point in time a large tumor could contain many potential new sub-clones with potential to evolve and some already forming sizeable sub-clones. Certainly, tumors show morphological differences in different regions and these can be accompanied by changes in protein expression detected by immunohistochemistry or other assays.

**MicroRNAs in Human Cancer**

MicroRNAs (miRNAs) are evolutionarily conserved small non-coding RNAs, ranging in length from 16 and 29 nucleotides. The miRNAs are postulated to form an endogenous system to regulate and coordinate the expression of genes on a post-transcriptional level [37,38]. They are able to bind complementary sequences in target messenger RNAs (mRNAs) and thus prevent their translation. Each miRNA may potentially target several hundreds different mRNA molecules, suggesting they may exert a one-step control over cellular processes [39]. The exact mechanism of the translational “silencing” is not known, but recently the target mRNAs were found to be sequestered in the so-called Processing Bodies (P bodies) distant from the translating ribosomes [40-42]. At the moment, more than 4,000 different miRNAs are identified or predicted in the genomes of viruses, plants and animals, of which some 700 may occur in man [43]. Some mammalian miRNAs are located within gene introns and appear to be transcribed within the primary transcript, only to be released during RNA processing [44]. In recent years, miRNAs have been shown to influence a variety of cellular processes of key importance, including cellular differentiation and maintenance of a differentiation state, developmental timing, proliferation and apoptosis [45,46]. Since deregulated cell death and proliferation are hallmarks of many types of carcinomas, it is not
surprising that, on the one hand, alterations in miRNA may lead to carcinogenesis, and, on the other hand, many miRNAs are found to be abnormally expressed in clinical cancer samples. The first study showing involvement of miRNA in human cancer was done by Calin et al., [47]. In search of a tumor suppressor gene in Chronic Lymphocytic Leukaemia (CLL) cases, they found that the smallest common lesion of a 30-kb region located at chromosome 13q14 coded for two miRNAs, miR15 and miR16. Furthermore, both genes were found to be deleted or downregulated in a majority (approximately 68%) of CLL cases. The discovery of a germ-line point mutation in two CLL patients that resulted in downregulation of both miRNAs and the induction of apoptosis by miR15 and miR16 by negatively regulating anti-apoptotic oncogene BCL2 in the leukemic cell line MEG-01 supported the putative tumor suppressor role of these miRNAs [48,49]. MiRNAs may also act in an oncogene-like manner. The amplification of the miRNA gene cluster miR-17-92 on chromosome 13 in human B-cell lymphomas leads to up regulation of several miRNAs that together with MYC oncogene accelerate tumor development [50]. Transcription of this cluster is induced by MYC itself. Similarly, overexpression of miR-155 in B-lymphocytes of transgenic mice leads to pre-leukaemic pre-B cell polyclonal expansion followed by B-cell malignancy [51]. Considering how rapidly data have been accruing in the last years, it is reasonable to believe that the next decade will bring new insights about the role of miRNAs in carcinogenesis and their therapeutic tools.

Changes shown are common deletions that may be identified by LOH analysis and different mutations in TP53 (TP53 mut 1 and 2). 9q-, 11p-, etc. denote deletions of the long (q) or short (p) arms of different chromosomes. Sub-clonal evolution within the population may lead to several distinct but spatially related tumours that differ in some but not all genetic changes. A final event in one cell may allow a metastatic clone to evolve.

Figure 5: Example of possible evolution of a metastatic tumor from a single initiated cell, over the course of many years.

Theories of Cancer

Multistage theory

Origins of multistage theory: Two different lines of thought developed the idea that cancer progresses through multiple stages. The first line arose from the observation that, in experimental animal studies, cancer often followed after sequential application of different chemical carcinogens. The second line arose from observations on the age-onset a pattern of cancer, in which incidence often accelerates with age in a manner that suggests multiple
Experimental Carcinogenesis: In the 1920s, several laboratories began to apply chemical carcinogens to experimental animals. Deelman [52] summarized observations in which repeated applications of tar to skin led to a small number of tumors, after which tarring was stopped. A few days later, the skin was cut where no tumors had appeared. Most incisions developed tumors in the scars; most such tumors were very malignant. Two distinct processes, tarring and wounding, combined to cause aggressive cancers. Twort and Twort [53,54] described several experimental protocols in which sequential application of different chemicals was much more carcinogenic than either agent alone. In the early 1940s, several others, notably Rous and Berenblum, reported similar observations on the co-carcinogenic interaction between two different treatments when applied sequentially [55-57]. Friedewald and Rous [58] described the first treatment as an initiator, because it seemed to initiate the carcinogenic process but were usually not sufficient by itself to cause cancer. They called the second treatment a promoter, because it caused progression of previously initiated cells but by itself rarely led to cancer. In a series of papers, Berenblum and Shubik [59-61] synthesized the experimental studies and thinking on co-carcinogenesis into the two-stage theory of initiation and promotion. The mechanistic action of initiators and promoters has been widely debated. In some cases, it was thought that the initiator is mutagenic; causing latent DNA lesions in some cells, and the promoter is mutagenic, stimulating cell division and providing favorable conditions for tumor formation. However, no simple mechanistic explanation fits all cases. Indeed, many observations from experimental carcinogenesis do not fit with a simple two-stage explanation [62]. The initial theory provided a useful framework for the early experimental studies, but hardened too much into “two-stage” and “initiator-promoter” slogans that probably hindered as much as helped to understand the actual mechanisms of carcinogenesis [62]. Recent emphasis has moved closer to the actual molecular mechanisms involved, aided by the great technical advances now underway. Aspects of initiation and promotion may play a role, but the older dominance of the rigid two-stage theory has naturally faded. For our purposes, the two-stage theory is important because it provided the first evidence and thinking with regard to multiple stages in cancer progression.

Age-specific incidence: Two observations about cancer incidence in epithelial tissues have led to multistage theories. First, cancer incidence often increases rapidly with age. Second, what happens to any particular individual appears to be highly stochastic, yet simple patterns emerge at the population level. In a rarely cited paper, Charles and Luce-Clausen [63] developed what may be the first quantitative multistage theory. They analyzed observations on skin tumors from mice painted repeatedly with benzopyrene. They assumed that benzopyrene causes a mutation rate, u, and that cancer arises by knockout of a single gene following two mutations, one to each of the two alleles. If t is the time since the start of painting with the carcinogen, then the probability of mutation to a single allele is roughly ut, and the probability of two hits to a cell is (ut)^2. They assumed that painting affects N cells, so that N(ut)^2 cells are transformed, and that the time between the second genetic hit and growth of the transformed cell into an observable papilloma is i. From these assumptions, the number of tumors per mouse after the time of first treatment is n = N[ut - i]^2. This formula gave a good fit to the data with reasonable values for the parameters. Thus, Charles and Luce-Clausen [63] provided a clearly formulated multistage theory based on two genetic mutations to a single locus and fit the theory to the age-specific incidence of tumors in a population of individuals. They assumed that both genetic hits must happen to a single cell, after which the single transformed cell grows into a tumor. Muller [64] mentioned the need for multiple genetic hits: “There are, however, reasons for inferring that many or
most cancerous growths would require a series of mutations in order for the cells to depart sufficiently from the normal.” However, Muller did not connect his statement about multiple genetic hits to age-specific incidence. The next theoretical developments followed directly from the observation that several cancers increase in incidence roughly with a power of age, $t^{n-1}$, where $t$ is age and the theories suggested that $n$ is the number of rate-limiting carcinogenic events required for transformation. Fitting the data yielded $n \approx 6–7$ distinct events. Whittomore and Keller [65] usefully separate explanations for the exponential increase of incidence with age between multi cell and multistage theories. The multi cell theory assumes that the distinct carcinogenic events happen to $n \approx 6–7$ different cells in a tissue [66]. If the carcinogenic events occur independently in the different cells, then this process would yield an age-specific incidence proportional to $t^{n-1}$, matching the observations. In particular, this theory leads to an expected incidence of

$$I(t) \approx (Nu)^n t^{n-1} / (n-1)!$$  \hspace{1cm} (1)$$

Where $N$ is the number of cells at risk for transformation, and $u$ is the transformation rate per cell per unit time; thus, $Nu$ is the rate at which each transforming step occurs in the tissue.

The multistage theory assumes that changes to a tissue happen sequentially. Charles and Luce-Clausen [63] explicitly discussed and analyzed quantitatively two sequential mutations to a particular cell; Muller [64] discussed in a general way sequential accumulation of mutations. Nordling [67] introduced log-log plots of incidence data to infer the number of steps. Nordling [67] assumed that the steps were sequential mutations to a cell lineage, and he suggested that a log-log slope of $n-1$ implied $n$ mutational steps in carcinogenesis. From data aggregated over various types of cancer, he inferred $n \approx 7$. Stocks [68] followed Nordling [67] with a mathematical analysis to show how sequential accumulation of $n$ changes to a cell leads to log-log incidence plots with a slope of $n-1$. Stocks [68] had the right idea, although from a mathematical point of view his analysis was rather limited because he assumed that changes happened at a constant rate per year and that at most one change per year occurred. Armitage and Doll [69] crystallized multistage theory by extending the data analysis and mathematical development. With regard to the data, they examined log-log plots for several distinct cancers rather than aggregating data over different cancers as had been done by Nordling [67]. With regard to theory, their mathematical model allowed different rates for different steps; they assumed continuous change rather than arbitrarily limiting changes to one per year; and they noted that the stages did not have to be genetic mutations but only had to be sequential changes to cells. The style of data analysis and mathematical argument formed the basis for the future development of multistage models. Armitage and Doll [69] rejected Fisher and Hollommon’s [66] multi cell theory in which the changes happen to different cells. Armitage and Doll argued that if a chemical mutagen caused cancer by causing mutations to several different cells, then incidence would increase with dose raised to a high power. For example, in Eq. (1), if the mutation rate, $u$, increases linearly with dose, $d$, then for $n$ steps in carcinogenesis, the incidence is proportional to $d^n$. In those cases known to Armitage and Doll, incidence increased only with a low power of dose but a high power of time. Thus, they rejected the multi cell theory. Against Armitage and Doll’s quick rejection of multi cell theory, Whittomore and Keller [65] pointed out that if a particular carcinogen affected only a few of the various stages in progression, for example only $m < n$ of the stages, then multi cell theory predicts that incidence would increase as $d^m$. So, Armitage and Doll’s argument did not really rule out the multi cell theory. Later molecular evidence tends to favor sequential changes to a cell lineage rather than changes to many different cells. However, recent work on genetic changes in stromal cells and analyses of the
tissue environment will probably lead to the conclusion that changes to the surrounding cells and tissue can also be important in some cases. The next step in the history, from a chronological point of view, concerns the role of cell proliferation and clonal expansion. However, I delay that topic until a later section. Instead, I take up what I consider to be the next major insight: how to test theories of progression.

**Cancer Stem Cell Hypothesis and Microenvironment**

Stem cells are pluripotent undifferentiated cells capable of undergoing a self-renewing cell division in contrast to embryonic stem cells that are omnipotent. The asymmetric division of a stem cell, by definition, yields one daughter cell that can differentiate along multiple lineages and a daughter stem cell with all the properties of the parental stem cell. A spectrum of cells with varying degrees of stamens is recognized by phenotypic markers. These cells are presumed to represent the second or third generation of stem cells that have undergone some preliminary commitment to one or more of the tissue lineages. Thus, a mesenchymal stem cell may differentiate to produce adipocytes, fibroblasts, osteoblasts and a host of other mesenchymal cells, but it is committed to the mesenchymal lineage. In 1926, Bailey and Cushing proposed that cancer was initiated and maintained by a subpopulation of transformed precursor cells. However, it was not until recently that Dick and co-workers [70] showed that only a few (0.1-1%) of the tumor cells present within an Acute Myeloid Leukaemia (AML) sample had the capacity to initiate AML growth after transplantation into NOD/SCID mice. Since then, small populations of cells with self-renewing capacity have been isolated from most leukemia’s, solid cancers such as medulloblastoma and glioblastoma, as well as carcinomas of different organs. These putative cancer stem cells are defined as cancer cells with stem-like properties, such as the ability to remain quiescent for long periods of time and the capacity for asymmetric cell division giving rise to one cancer stem cell and one differentiated progeny. However, they differ from normal stem cells by demonstrating unregulated proliferation, probably due to acquired gene mutations that render them less responsive to negative growth signals or to the loss of contact inhibition and gap junction intercellular communication. They display the same cell surface markers as their normal tissue counterparts, allowing their isolation and enrichment. The definition of cancer stem cells directly implies that a cancer treatment can only be successful if all cancer stem cells are killed. A subset of cancer stem cells expresses multidrug resistance transporters ABCB1 and ABCG2 [71]; others express constitutively Vascular Endothelial Growth Factor Receptors (VEGFR2) and seem to be the source of intrinsic vasculature building for the tumor [72]. Studies with glioblastoma and breast cancer stem cells indicate an increased radio resistance due to a more efficient DNA damage repair compared to non-stem cancer cells [73]. The development of approaches to radiosensitive tumor stem cells remains an important future challenge. Although many fruitful studies on cancer biology have been performed in monotypic cell culture, the basic structural unit of living tissues remains a highly complex three-dimensional mixture of cell types. In 1959, Letterer defined the morphology of this complex mixture as a histone; more recently, the term microenvironment has been used. It is important to note that the microenvironment includes not only different cell types, such as fibroblasts, endothelial cells, tissue macrophages, leucocytes, nerve cells, etc., but also extracellular matrix, serum and lymph proteins, and a whole host of locally and systematically acting secreted molecules. Within the microenvironment, tumors develop and interact with the different components. It seems unwise to assume that tumor stem cells are immune from the influence of this microenvironment [74].

**Radiation-Induced Cancers**

Ionizing radiation is an effective carcinogen, causing malignant transformation of
many different tissues. The shape of the dose-response relationship for cancer induction is currently assumed to be best represented by a linear no-threshold relationship. This also describes the dose response observed for the accumulation of damage to cellular macromolecules, in particular DNA. Although not universally accepted, it is considered that a failure to repair DNA damage leads to the permanent accumulation of gene mutations in irradiated tissues that then lead to alterations in the regulatory pathways described above. Alternative views give more weight to non-targeted effects of radiation damage, including local inflammatory and stress responses, which are postulated to lead to more global changes in mutational activity characterized by genomic instability. Evidence for a direct, targeted, mutational event in radiation-induced cancers is lacking, even for alpha-radiation, which would be expected to induce characteristic large deletions in critical genes, which should then be present in all progeny cells. A number of studies have reported either specific gene alterations (e.g. RET/PTC3 translocations in radiation-induced thyroid cancer, AMLETO alterations in radiation-associated myeloid leukemia) or a specific profile of gene expression changes (e.g. in radiation induced osteosarcoma and papillary thyroid cancer). However, the specificity of these changes may reflect the histopathologic uniqueness of the radiation-induced tumors, which suggests that they may be derived from different progenitor cells than those giving rise to sporadic cancers in these tissues. An additional complication is that many radiation-induced cancers arise in genetically predisposed individuals who have inherited a germ-line mutation (e.g. in the RB1 gene). The mechanisms behind the development of therapy-associated cancers in such an individual may well be quite different from those in sporadic cancers.

Conclusion

The underlying molecular mechanism responsible for the development of a tumor cell may vary (e.g. inactivation of a tumor suppressor gene by a virally encoded protein, inheritance of a germ-line mutation or sporadic point mutation of an oncogene). Nevertheless, all of the mutational events target a common set of regulatory nodes within the cell, such as the cell cycle checkpoints, growth factor independence and prevention of apoptosis. The wide spectrum of genetic alterations, even within one tumor type, reflects the multiple points at which key processes may be subverted and camouflages a much more simple biological process involving only a set of critical processes.

Evolving concepts of tumor stem cells, the regulation of coordinated expression programmes by non-translated microRNAs and the role of the tumor microenvironment are just three areas where new knowledge is opening up possibilities for the diagnosis and treatment of malignant disease. In all three situations, the role of ionizing radiation is, at best, poorly understood, and harnessing them for therapeutic purposes requires that considerable effort be expended to define their interaction with radiation.

References


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Introduction

Endocrinology is relatively a new branch of physiology began with classic experiments of a French scientist Ernald Berthold (1849) on domesticated rooster [1]. William Bate Hardy (GB) is credited with coining the term hormone while visiting in the laboratory of Bayliss and Starling [2]. Hormones are chemical messengers pass through the blood and communicate signals from one type of cells to another and affect many vital processes [3]. Almost all the physiological system is regulated by these secretary molecules. Presently endocrinology comes up in existence with the emphasis of structural, functional, molecular and pathologinal knowledge of hormone and system related to the regulation. Hormone acts on target cell via specific protein receptors located either on the cell surface or in the cytoplasm or nucleoplasm [4]. Hormones–receptor complex initiate a series of molecular events through signal transduction which ultimately influence the function of cell [4].

On the basis of chemical nature, hormones are classified into three groups: amino acid derivatives, peptide hormones and lipid derivatives (steroid hormones and eicosanoids) [5]. These are different in their molecular structures and mechanism of action. Steroid hormones are secreted by the adrenal cortex and the reproductive glands. They have appreciable binding affinity with receptors present in the nucleus or in the cytoplasm of the cells and form hormone receptor (HR) complexes [6]. These HR complexes bind to the hormone response element, a region of the DNA of cells and exert a positive or negative effect on target gene transcription. Amino acid or peptide hormones are produced by the thyroid gland and adrenal medulla [7]. These hormones interact with the receptors that are already associated with specific DNA regions [8]. The interaction transforms the action of the affected genes and ultimately leads to cell type-specific responses. Several glands such as hypothalamus, pituitary gland, pancreas and parathyroid gland secretes polypeptide and protein hormones. These hormones interacts with the receptors present on surface of cell membrane and thus, initiates the function of cells.

Secretions of hormones are very vital for achieving an optimal biological functioning and
Whenever there is excess or lesser secretion of a particular hormone, endocrine disorders arises. The unwanted growth of tissues or cells that affects the parts of the endocrine glands that secrete hormones are known as endocrine tumor. Endocrine tumor starts in the cells that produces hormones, so that the tumor itself can make hormones and cause severe imbalance in hormone system and illness [9]. The main type of endocrine tumors is described as follows.

**Adrenal Gland Tumor**

Adrenal glands also called suprarenal glands are found in pairs on the top of each kidney [1,2]. It is triangular in shape, weighs about 5 g and measures about 50 mm in length, 30 mm wide and 10 mm thick [11,12]. The outer yellowish portion of the adrenal gland is called the adrenal cortex and inner reddish portion is known as adrenal medulla [11,13]. Adrenal cortex is divided mainly into three regions, first one is zonaglomerulosa, outer layer of adrenal cortex which produces mineralocorticoids mainly aldosterone, second one is zona fasciculata which secretes glucocorticoids popularly cortisol and the third one is zonareticularis which is the innermost layer secretes androgens, mainly dehydroepiandrosterone(DHEA), and androstenedione(the precursor to testosterone sex hormone) [14]. Aldosterol hormone regulates salt and water balance in the body, and cortisol regulates carbohydrate, protein, and fat metabolism in the body [15]. During stress conditions [16] adrenal medulla produces hormones called catecholamines such as adrenaline (also called epinephrine) and noradrenaline hormone (also called norepinephrine) [17]. These hormones are responsible for faster heart beat, sweating, increase in blood supply and also cause widening of the eyes [16].

A tumor can develop either in the cortex or medulla part of the adrenal gland and also it is possible that it can be cancerous (malignant) or non-cancerous (benign) [18,19]. Generally tumor is found in only one gland and rarely both gland are affected known as bilateral adrenal tumors. Some adrenal gland tumor produces the excess amount of hormones and therefore, shows dramatic symptoms, because of these reasons they are known as functioning tumors [19,20]. On the other hand, some adrenal gland tumors are called non-functioning because they produce no hormones, and therefore they do not show any obvious symptoms [19,20]. Benign tumors and malignant which are developed in adrenal cortex part are known as adrenal cortical adenomas and adrenal cortical carcinomas respectively [20].

**Adrenal cortical adenomas**

These are the most common type of adrenal gland tumors but uncommon in patients younger than 30 years old. Several advance technologies are utilized to treat cancer such as chemotherapy, radiation therapy and hormonal therapy may also be employed in the treatment of this disease, including surgery and thermal ablation [20-22]. It is generally non-functioning tumor and therefore, usually does not show any symptoms in the early stages [22]. However, some adrenal cortical adenomas are functional tumors and overproduce several hormones such as mineralocorticoids, glucocorticoids and androgens which cause several endocrine disorders such as Conn’s syndrome (hyperaldosteronism, overproduction of Aldosterone hormone), Cushing’s syndrome (overproduction of glucocorticoids), virilization of females, or the feminization of males [22-26]. Symptoms of Conn’s syndrome include high blood pressure, low potassium level in the blood, hypertension, weakness, muscle cramps, increased thirst and frequent urination [24-28]. Surgical adrenalectomy and laparoscopic adrenalectomy methods are used to remove the adrenal gland to treat Conn’s syndrome [27-29]. Cushing syndrome (also called Itsenko-
Cushing syndrome, hyperadrenocorticism or hypercorticism) show several symptoms, including high blood pressure, impaired glucose tolerance or diabetes, hyperlipemia and osteoporosis, weakness in the legs, depression, fat deposit behind the neck and shoulder, hair growth on face, chest and back in women, purple stretch marks on the stomach and neck and central obesity [23,29-33]. Removal of the adrenal gland is one of the options to treat Cushing syndrome [30,31,33]. Several drugs, including ketoconazole, metyrapone, mitotane, or etomidate, cabergoline, octreotide, and mifepristone are used to treat Cushing syndrome [33].

**Adrenocortical carcinoma**

It is a rare and highly aggressive form of cancer found in cortical cells of the adrenal gland [20]. It can be a functional or non functional tumor depending on the secretion of hormones [20,35]. It can show similar symptoms to the adrenal cortical adenomas, but sometimes it might be different [20,35]. It can develop at any age, but accumulating data suggested that it is more frequent in adults whose age is 40-50 years [36]. Surgery of adrenal gland is most preferred options to treat adrenocortical carcinoma, although several other treatments are also given along with this such as chemotherapy, and radiofrequency ablation [37].

**Pheochromocytoma**

It is an endocrine tumor found in the medulla of the adrenal gland and overproduce catecholamines, usually epinephrine (adrenaline) and norepinephrine (noradrenaline) [23]. Because of excess secretion of the hormone, they show numerous symptoms such as high blood pressure, headache, palpitations, anxiety attacks, excessive sweating, weight loss, and pallor [23,38]. This tumor can be found in patients of multiple endocrine neoplasia and Von Hippel–Lindau (VHL) [39-41]. It can be diagnosed by measuring the concentration of cortisol and catecholamines or metanephrines in the blood or urine. Advance Imaging technology such as ultrasound, magnetic resonance imaging or computed tomography of head, neck, chest or abdomen can be used to localize the tumor [43].

**Neuroblastoma**

It is a very common cancer often found in children and originates in the medulla part of the adrenal gland [44]. Moreover, it is also an aggressive cancer of immature neuroblastic cells and sometimes do not show any symptoms [45]. Common symptoms of this disease are loss of appetite, fever, fatigue and joint pain. It can show swollen belly and constipation in the abdomen, breathing problems, bruising and swelling of eyes and weakness [44,46]. Neuroblastoma also secretes hormones, causing symptoms such as constant diarrhea or high blood pressure [47]. This tumor is generally diagnosed by biopsy and also the level of catecholamine hormone is measured in the urine for the diagnosis. Moreover, blood and urine test, molecular genetic studies, computed tomography (CT) scan, positron emission tomography (PET) scan, magnetic resonance imaging (MRI), and bone scans are the methods utilized to diagnose this disease [44,46].

**Adrenal incidentalomas**

Adrenal incidentaloma is a mass lesion, generally 1 cm or more in diameter, discovered incidentally by advance imaging such as computed tomography (CT), magnetic resonance imaging (MRI), or ultrasonography [48]. In case of older, obese, diabetic, and hypertensive patients, the prevalence of adrenal incidentaloma is higher [49,50]. These masses are may be non-functional (do not secrete hormone) or functional and also may be malignant or benign in nature [49,50] (Figure 1).
For the functional tumors, adrenalactomy is required to remove the gland and tumor. In case of non-functional tumors, the size and imaging characteristics of the tumor as well as the patient’s age and other health problems will determine for the adrenalactomy. If the operation is unsuitable for the patient, then observation or close follow-up is the best treatment to see if the tumor becomes functional. The risk that a tumor will become hyperactive is greatest in the first -4 years which can develop in to Cushing’s syndrome.

**Gastrointestinal Tumor**

The gastrointestinal tract is part of body digestive system, which helps to digest food and also help in excreting the waste material from the body [51]. Stomach, small intestine, large intestine (colon and rectum) are main parts of the gastrointestinal tract. It is very well documented that certain type of neuroendocrine cells is found in the gastrointestinal tract which produce hormones to help in moving food through the stomach to intestine [51]. In these types of neuroendocrine cells gastrointestinal carcinoid tumor can begin which can also make hormones and release them into the body [58]. These types of tumor are very rare and slow growing which can occur in the appendix, small intestine and rectum. The person who has a family history of multiple endocrine neoplasia type 1 (MEN1) syndrome or neurofibromatosis type 1 (NF1) syndrome are more prone to gastrointestinal tumor [51]. Moreover, the person suffering from atrophicgastritis, pernicious anemia, or Zollinger-Ellison syndrome is also more prone to gastrointestinal tumor [59]. Most of the time initially they do not show any symptoms but later on when they spread they can show many symptoms. Release of serotonin and other substances from the tumors of gastrointestinal tract show many symptoms including flushing of the face, abdominal pain, diarrhea, fast heartbeat, bronchial spasms and sudden drops in blood pressure as mentioned-above [60]. Several lab tests and imaging technology are used to detect and diagnose cancer including blood and urine test, tumor marker test, magnetic resonance imaging (MRI) PET scan (positron emission tomography scan), CT scan, endoscopic ultrasound, colonoscopy, capsule endoscopy and biopsy [61]. Several treatment options are available for patient suffering from a gastrointestinal tumor such as surgery, radiation therapy, chemotherapy and hormone therapy [51].
Lung Carcinoid Tumor

We know that lungs also contain neuroendocrine cells, which secrete hormone and they help to detect the level of oxygen and carbon dioxide in the breath air and release chemical to send the message to adjust these changes [58]. The tumor which develops in these neuroendocrine cells are known as a lung carcinoid tumor, which is extremely uncommon and grow very slowly as compared to other types of lung cancer [59,62]. There are following four types of lung carcinoid tumors.

Small cell lung cancer

It is one of the fastest growing cancer cells, which multiplies quickly and spread to other organs such as lymph nodes, bones, brain, adrenal glands, and liver. It spread so early and due to this reason, chemotherapy is a better choice to treat this as compared to surgery [63].

Non-small cell lung cancer

There are three types of non-small cell lung cancer, squamous cell cancer, adenocarcinoma and large cell carcinoma. Squamous cell cancer and adenocarcinoma develop from the cells that line the airways of the lungs. Large lung cell carcinoma is called because they appear larger in size under the microscope and also it is well documented that these types of cells grow very quickly [64,65].

Typical lung carcinoid tumor

Typical lung carcinoid tumor, also known as typical pulmonary carcinoid tumor, is a slow growing tumor which rarely spread to other organs. It is typically present with cough or hemoptysis. They are diagnosed by microscopic examination and treated by surgical excision [58].

Atypical carcinoid tumor

Atypical carcinoid tumor is faster and can spread to other organs as compared to typical carcinoid tumor [58].

Pancreatic Tumor

The pancreas is a gland found in the abdomen and also an integral part of both digestive and endocrine system, which secretes hormone to regulate the body and also digestive enzymes to break down food into smaller parts [66]. There are mainly two types of cell, exocrine and endocrine, which are found in the pancreas. Exocrine pancreatic cells produce enzymes and release it into the small intestine which helps in digestion of food, particularly fats [67]. On the other hand, endocrine pancreatic cells, also known as islet cells or islets of Langerhans, secrete several type of hormones including insulin, which helps in controlling the blood sugar level in the body [66-68].

It is well established that pancreatic cancer is one of the major causes of cancer death in the world [69]. As mentioned above two types of cells are found, therefore, pancreatic cancer is categorized into two group adenocarcinoma, which begins in exocrine component and pancreatic neuroendocrine tumor, which begin in the endocrine component [70,71]. The adenocarcinoma is a common form of pancreatic and it is a major cause of pancreatic cell death while as neuroendocrine tumor is very rare, comprise only 1 to 5% of all pancreatic cancers [72].
**Pancreatic neuroendocrine tumors**

Pancreatic neuroendocrine tumors can also be called a pancreatic islet cell tumor, pancreatic endocrine tumor or islet of Langerhans tumor [70,71]. Generally following types of islet cell tumors are classified.

Nonfunctioning tumors: Pancreatic neuroendocrine tumors are generally nonfunctioning tumors which do not show hormonal symptoms, and therefore, they are usually diagnosed at more advanced stages of disease [73].

Functioning islet cell tumors: These tumor cells show dramatic symptoms because of excess secretion of different types of hormone into blood [73,74]. They are classified into five types based on the hormone normally produced by the cells.

- **Gastrinoma**: These tumor cells produce excess amount of gastrin, which produces acid in the stomach which can cause severe ulcer [75].
- **Insulinoma**: These tumor cells produce excess amount of insulin causing hypoglycemia (low blood sugar) [76].
- **Glucagonoma**: These tumor cells produce excess amount of the hormone glucagon causing hyperglycemia (High blood sugar) [77].
- **VIPoma**: It is an extremely rare islet cell tumor that produces excessive amounts of vasoactive intestinal peptide (VIP), which can cause watery diarrhea, called Verner-Morrison syndrome [78].
- **Somatostatinoma**: It is also an extremely rare tumor that produces excessive amounts of somatostatin, which stops the secretion of several other hormones, including growth hormone, insulin, and gastrin [79].

**Symptoms of pancreatic cancer**

Non functional endocrine tumor usually takes longer time to spread other parts of the body without showing any symptoms. Later, when they grow and spread into many parts of the body they can show several symptoms, including indigestion, diarrhoea, pain in abdomen or back, a lump in the abdomen, whites of the eye and yellowing of skin [80]. Functional endocrine tumor secretes several types of hormones which show dramatic changes in the body and can show several symptoms depending on types of tumors. Insulinoma can show symptoms such as low blood sugar which can cause headache, blurred in the vision, tired, weak, shaky, nervous, irritable hungry and fast heart beat while as glucagonoma can show symptoms such as high blood sugar which can cause frequent urination, headaches, dry skin and mouth, or feeling thirsty, hungry, weak or tired [81]. It can also show other symptoms including skin rash on the stomach, face, or legs and blood clots which can cause shortness of breath and cough in the lung or pain in the chest and also can cause swelling, pain, warmth, or redness in the arms or legs [81]. Somatostatinoma can show symptoms such as high blood sugar which can cause frequent urination, headaches, dry skin and mouth, or feeling thirsty, hungry, weak or tired. It can show other symptoms such as diarrhea, gallstones, weight loss, whites of the eyes and yellowing of the skin [81]. Furthermore, gastrinoma can show symptoms such as stomach ulcer, pain in abdomen and diarrhoea [70,83,84]. VIPoma can cause diarrhea, dehydration, low potassium level in the blood, cramps or pain in the abdomen and weight loss [83,84].

**Detection of pancreatic tumor**

Several techniques and imaging technologies are available to detect and diagnose...
pancreatic cancer [85,86]. Moreover abdominal CT scan, magnetic resonance imaging (MRI), somatostatin receptor scintigraphy, endoscopic ultrasound (EUS), endoscopic retrograde cholangiopancreatography (ERCP), angiogram, laparotomy, intraoperative ultrasound, biopsy and bone scan lab tests/imaging techniques are available to detect and diagnose pancreatic cancer [71,87-90].

Other type of lab tests to detect different types of pancreatic endocrine tumor are as follows [70,71,75,91,92].

Test for gastrinoma tumor: (a) Secretin stimulation test, (b) Fasting serum gastrin test, (c) Basal acid output test, (d) Somatostatin receptor scintigraphy.

Test for insulinoma tumor: Fasting serum glucose and insulin test

Test for glucagonoma tumor: Fasting serum glucagon test

Test for VIPoma tumor: (a) Serum VIP (vasoactive intestinal peptide) test, (b) Stool analysis, (c) Blood chemistry studies.

Test for somatostatin: (a) Fasting serum somatostatin test, (b) Somatostatin receptor scintigraphy.

**Treatment of pancreatic tumor**

Nowadays, several advanced technologies are available to treat this disease. The most common option is surgery, however many other options are also widely used such as radiofrequency ablation, chemotherapy, interferon, transplantation, and angiographic chemoembolization [92-94].

**Parathyroid Tumor**

Parathyroid glands are four small endocrine glands located in the neck region of our body [95]. These glands secrete a hormone called parathyroid hormone (PTH) which regulates calcium and phosphorus levels in the blood [95-97]. Over secretion of parathyroid hormone called hyperparathyroidism caused several problems, including osteoporosis, ulcers, pancreatitis, kidney stones and mental disorders [95,98]. Generally, the overgrowth tissue which secretes excess amount of PTH hormone is non malignant known as parathyroid adenomas (benign parathyroid hormone secreting tumors) [99,100]. In most of the patients, only one parathyroid gland in affected and in a few cases, two or three glands are affected and in extremely rare cases, all four glands are affected [95].

**Symptoms of parathyroid tumor**

Moreover, in general, parathyroid cancer is very rare, and it secretes huge amount of PTH hormone, which mobilizes huge amounts of calcium (this condition is called hypercalcemia) from the bones, releasing this calcium into the blood stream [101]. People suffering from multiple endocrine neoplasia type 1 or autosomal dominant familial isolated hyperparathyroidism or hyperparathyroidism-jaw tumor syndromes are more prone to Parathyroid Tumor [102].

**Detection of parathyroid tumor**

Parathyroid tumor can be detected by measuring calcium and PTH level in the blood [103]. Neck ultrasound and sestamibi scan are generally done to detect that which parathyroid gland is abnormal [104,105].
Treatment of parathyroid tumor

This cancer can be treated by surgery of the parathyroid glands, and generally, radiation therapy is used to kill cancer cells [106,107]. The reports show that usually chemotherapy is not used for treating this disease because presently no effective drug is available in the market [107].

Pituitary Gland Tumor

It is a small gland found inside the skull, just above the passage of the nose, and it is also known as the master gland because it secretes several hormones which control many endocrine functions [108]. It is connected directly to part of the brain known as the hypothalamus. The pituitary gland is divided into two parts, anterior lobe and posterior lobe [108-110]. The anterior pituitary is larger front part of the gland secretes different hormones. Growth hormone (GH) or somatotropin is mainly secreted in children to promote body growth during childhood [109,111]. A very little amount of GH is found in adults and the condition in which an adult makes too much GH is known as acromegaly. Thyroid-stimulating hormone(TSH)or thyrotropin promotes the release of thyroid hormone and growth of the thyroid gland [109,112]. Adrenocorticotropic hormone (ACTH) or corticotropin promotes the release of steroid hormones and growth of the adrenal glands [109]. Luteinizing hormone (LH) or lutropinpromotes and help in development of the corpus luteum [113]. Follicle-stimulating hormone (FSH) stimulates the growth of immature ovarian follicles in the ovary [114]. Prolactinpromotes milk production in women [115]. The smaller back part of the pituitary gland is known as the posterior lobe which secretes mainly two hormones Oxytocin and Vasopressin. Oxytocinpromotes to release milk from breast and also help the women to contract her uterus during child birth [116]. Vasopressin is also called antidiuretic hormone (ADH), helps to retain water in the body through increasing water absorption in the collecting ducts of the kidney nephron. Moreover, it also plays a role in constricting the blood vessels in the body [117].

The tumor can develop in the pituitary gland region. It can be either functional or non-functional tumor. The functional tumor overproduces the hormones whilst non-functional tumor does not produce hormones [118-121]. The pituitary gland tumor shows several general symptoms, including headache, loss of vision, weight loss, Cushing's syndrome, acromegaly, sweating and infertility [109,118]. Generally pituitary tumors are benign (non-cancerous), but sometimes they are cancerous also. They are classified into pituitary adenomas, invasive pituitary adenomas and pituitary carcinomas.

Pituitary adenomas

These are benign pituitary tumors, which are non-cancerous and do not spread outside the skull. They remain within the skull and do not have enough space to grow. Depending on the size of the tumor they are divided into microadenoma and macroadenoma. Microadenoma are smaller than 1 centimeters (cm) across and sometimes very difficult to detect them because of their smaller size while macroadenoma are equal to or greater than 1 centimeter (cm) across and detected easily because of the larger size [109,118,119]. Most of the pituitary adenomas are microadenoma and often discovered incidentally known as incidentalomas [119]. They are often discovered during the examination of patients for the unrelated conditions, for example, headache and dizziness [122]. Sometime pituitary adenomas grow in an abnormal place, most particularly in the sphenoid sinus, suprasellar region, nasopharynx and the cavernous sinuses region, referred as an ectopic pituitary adenoma [123,124].
Invasive pituitary adenomas

These tumors are generally not malignant. We know that pituitary adenomas are slow-growing tumors, which are found within the sellaturcica region of the brain, and when become aggressive and infiltrate the dura mater, cranial bone, or sphenoid sinus region, they are referred as invasive pituitary adenomas [125-127]. Usually surgery and radiotherapy techniques are used for treating this disease [110].

Pituitary carcinomas

Only few pituitary tumors are malignant and grow in other parts of the body. They spread to other parts of central nervous system and sometimes even spread outside the central nervous system [109,110].

Currently pituitary tumors can be diagnosed by utilizing several modern techniques and lab tests, including blood tests, Computed tomography (CT) scan, biopsy, lumbar puncture (spinal tap), and magnetic resonance imaging (MRI) [110,128]. Several types of treatment options are available for patients suffering from pituitary tumors such as surgery, chemotherapy, and radiation therapy [128,129].

Other sporadic pituitary tumors

Despite of the above types, there are many other benign as well as some malignant pituitary tumors which are much less common than adenomas. Out of these, teratomas, germinomas, and choriocarcinomas are some exceptional tumors that usually occur in children or young adults. Gangliocytomas and Rathke cleft cysts are uncommon tumors of pituitary more often than not found in adults. Craniohypophysealomas are more common in children but exceptionally in older adults. These are slow-growing tumors that start above the pituitary gland and compressing hypothalamus as well as the pituitary gland, causing hormonal tribulations. They Cancer that starts in sites other than the pituitary can metastasize to the pituitary and are not classified as pituitary tumors [109] (Figure 2).

Thyroid Tumor

It is an endocrine gland which is found just above the voice box and contain two parts or lobes (right and left lobes) and made up of two types of follicular cells, which produces thyroid hormone (thyroxine, T4 and triiodothyronine, T3), which is important for normal
functioning of the body and C cells, which produces calcitonin, a hormone that helps in calcium metabolism [130]. Thyroid gland needs a sufficient quantity of iodine, which is required for normal functioning of the thyroid gland [131]. Iodine is found in the food, including seafood and dairy products [131]. Overproduction of thyroid hormones is known as hyperthyroidism, and low production of thyroid hormones is known as hypothyroidism or myxoedema [132]. Losses of weight, feeling hungrier, shaky, anxious and faster heart rate are some symptoms associated with hyperthyroidism and in case of hypothyroidism weight gain, tiredness and lethargic are some symptoms associated with it [133,134]. If the level of thyroid hormone decreases in the blood, hypothalamus releases thyroid-releasing hormones into the blood which activates the pituitary gland to produce a thyroid stimulating hormone, and this thyroid stimulating hormone stimulates the thyroid gland to produce more T3 and T4 thyroid hormones [135,136].

Thyroid cancer is uncommon, but it is more common in women as compared to men [137]. Usually in men it is found in older age and very rare in children but in women, it is found in younger age [137,138]. Thyroid cancer is categorized into following types [139-142].

**Papillary thyroid cancer (PTC)**

It is one of the most common types of thyroid cancer generally found in younger women and develops from the follicular cells and grows very slowly. Out of all the thyroid cancers, 80-85% are PTC.

**Follicular thyroid cancer (FTC)**

It is less common and develops from the follicular cells and grows slowly. Both papillary and follicular thyroid cancers are differentiated thyroid cancer. FTC makes up about 7-15% of all the thyroid cancers.

**Medullary thyroid cancer (MTC)**

This is a rare type of cancer develops in C cells and persons suffering from multiple endocrine neoplasia type 2 (MEN2) are more prone to this disease. About 3 % to 5% of all the thyroid cancers are MTC type.

**Anaplastic thyroid cancer (ATC)**

This is very rare, fast growing and poorly differentiated thyroid cancer affecting 1-2% of all the thyroid cancer patients.

**Thyroid lymphoma (TL)**

This is the rarest (<0.5%) type of thyroid tumor found mainly in female, where non-Hodgkin’s B-cell lymphomas is found in most cases and also Hodgkin’s lymphoma is identified in few cases. The causes of thyroid cancer in most of the patients are unknown but there are some risk factors, which increase the chance of developing cancer such as benign thyroid disease, exposure to radiation, inherited faulty gene and weight gain [136,143]. Several advance technology and laboratory tests are available to detect and diagnose thyroid cancer such as the blood test (To detect T3, T4 and calcitonin hormone), ultrasound thyroid scan, biopsy, magnetic resonance imaging (MRI), computerized tomography (CT) scan, positron emission tomography (PET), vocal card check, and full body scintigraphy using iodine-131 [136,144-146]. Several types of treatment options are available to treat thyroid cancer. Usually surgery is the first choice to treat thyroid cancer in which thyroid gland is removed from the body [147]. Also hormone therapy and radiotherapy is used to treat this disease, however chemotherapy is generally not used for treatment purposes [148,149].
Concluding Remarks

Tumors associated with the hormone secreting glands are known as endocrine tumors which is one of the dreadful diseases in the world and millions of people are dying each year because of this. In this regard, cancer is a curable disease, if detected at an early stage, but at metastatic stage, this disease becomes very dangerous and also becomes less chance to cure this malady. Several modern technologies and tools have been developed in the last few years to diagnose this disease but even though we are unable to diagnose this disease at an early stage. Therefore, extensive research is required to develop cancer diagnostic tools, which should be very effective for the detection of tumors. at an early stage.

The most important point is the treatment strategies which are utilized to cure this disease. Nowadays, chemotherapy, hormonal therapy and surgery are generally used for treatment. Surgery is one of the most common options used to treat this disease at the beginning, but in many cases these approaches are not very much successful, thus alternative strategies are required for this. In many tumors, chemotherapy is not very much successful and this demand development of novel anticancer agents which could be applicable for this. We need to work in a distinct way to develop new therapeutic tools for cancer. In addition, the treatment strategies which are available currently is very costly and not affordable by the patients belongs to the developing or undeveloped countries. Low cost treatment strategies should be available for the patients and for this, we need to develop novel cheap anticancer drugs and other low cost diagnostic tools required for treating this disease. Now the time has come that awareness and valuable information regarding cancer should reach to the common people so that they should go for health checkup regularly.

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Introduction

It is now some 20 years since it became widely appreciated that the growth of solid tumours requires a functional vasculature and that disruption of this functional vasculature could lead to novel anti-cancer therapies. This has led to the development of many anti-angiogenic drugs, the first of which are now completing clinical trial. The purpose of this chapter is to review our current understanding of tumour angiogenesis and what the prospects are for anti-angiogenic therapies.

Angiogenesis

Solid neoplasms, including epithelial cancers, require the formation of a supporting stroma and vascular supply if they are to grow beyond the size of 1–2 mm (Figure 1). Epithelial cancers induce the formation of stroma, which is a complex Extracellular Matrix (ECM) rich tissue that comprises three main components. These include newly formed blood vessels (which supply the tumour with nutrients and also provide a means for gas exchange and waste disposal), activated fibroblasts, and inflammatory Infiltrates such as lymphocytes and macrophages, and a complex network of matrix proteins (Figure 1) [1]. Angiogenesis involves the formation of new capillary micro vessels from pre-existing parent vessels. This process is extensive during embryonic development, but restricted in normal adults, occurring only during the female reproductive cycle, wound healing, and in certain disease states such as psoriasis, diabetic retinopathy, and cancer [2]. In the developing embryo, two distinct processes give rise to the embryonic vascular network: vasculogenesis and angiogenesis. Vasculogenesis gives rise to the embryonic blood vessels such as the heart and the first primitive vascular plexus, while angiogenesis is responsible for subsequent remodeling and expansion of the network [3,4]. Under normal conditions, Endothelial Cells (ECs) seldom divide. However, during angiogenesis, quiescent ECs of the post-capillary and small terminal venules are stimulated by a variety of cytokines and growth factors to begin a cascade of events leading to the formation of new vessels. These include proteolytic...
degradation of the basement membrane, an event closely followed by EC proliferation, directed migration, and differentiation, giving rise to the formation of a lumen or sprout. Newly formed sprouts anastomose with one another, as well as with pre-existing vessels to form a hollow tube through which blood flow starts [5]. The newly formed endothelial vessels have been shown to lack cellular differentiation and to have a markedly different morphology from normal vessels. They acquire support from pericytes and a basement membrane, although this is often incomplete, resulting in leaky blood vessels from which protein macromolecules extravagate into the surrounding stroma [6]. They also undergo alterations in their cell surface expression of adhesion molecules, receptors, and other proteins, characteristically expressing unique antigenic markers such as the VEGF (vascular endothelial growth factor receptor) receptors, integrin’s such as avb3 [7], Delta-4 [8], and discovered EC-specific the recently magic roundabout protein [9-11]. While angiogenesis is believed to result exclusively from the proliferation, migration, and remodeling of fully differentiated ECs, vasculogenesis refers to the formation of embryonic blood vessels from EC progenitors or angioblasts [12]. However, in a study by Asahara et al. (1997) [3] isolated human EC progenitors from peripheral blood were capable of differentiating into mature ECs both in in vitro and in vivo experiments, incorporating into sites of active angiogenesis. Later studies showed that circulating EC progenitors were mobilized in response to tissue ischemia or cytokine therapy, thereby augmenting neovascularization of ischemic tissues [13]. These findings imply that post-natal vascularization (angiogenesis) does not rely exclusively on sprouting from pre-existing blood vessels. Instead bone marrow derived EC progenitors may also contribute to angiogenesis [3,13,14]. Studies by Lyden et al. (2001) [15], have also confirmed that endothelial progenitor cells arising in the bone marrow are active in tumor angiogenesis and are significant contributors to tumor vasculature. These findings arose from work in which it was found that adult mice that expressed reduced levels of the transcription factor Id were unable to support tumor angiogenesis [16]. The Id proteins interact with other transcription factors to modulate cellular differentiation in early fetal development. It was subsequently shown that transplantation of β-galactosidase expressing wild type bone marrow into lethally irradiated Id-mutant mice was sufficient to restore vascularization of implanted tumors [15]. If tumors are achieving substantial vascularization by recruitment of circulating endothelial progenitors this has profound implications for the development of new strategies to block tumor angiogenesis.

Figure 1: Epithelial tumours are comprised of malignant epithelium and stroma. The stroma includes tumour endothelium, activated fibroblasts, inflammatory infiltrates, and matrix proteins.
The tumour stromal compartment

Neovascularization is accompanied by the formation of a supporting stromal network in tumour lesions, which often makes up between 20 and 50% of the mass of a solid tumour (Figure 1). Depending on the type of neoplasm, the amount and composition of the stroma varies and in some cancers may contribute to more than 90% of the mass of the tumour [17]. The ECM of tumour stroma comprises fibres including collagen and elastin, a fibrin gel matrix, and glycoproteins such as fibronectin. Activated fibroblasts synthesize and secrete many of these ECM proteins, in addition to a variety of proteolytic enzymes and their inhibitors, which exert control over the composition and renewal of the ECM. The formation of the tumour stromal compartment closely resembles that of granulation tissue in wound healing [18]. Stromal breakdown has been associated with malignant growth, with metastasis resulting from the degradation of basement membranes, and the ECM of tumours by proteolytic enzymes such as collagenases, and other metallo- and serine-proteases. Fibroblasts, in addition to macrophages and mast cells, appear to be involved in the synthesis and regulation of these enzymes. Studies indicate that malignant tumour cells release factors that stimulate fibroblasts to synthesize enzymes, suggesting a close cooperation between the tumour and stromal cells in the regulation of proteolysis [19]. Activated stromal fibroblasts are involved in a number of physiological processes, including desmoplastic reactions during neoplasia, fibromatoses (non-malignant fibroblastic transformations), wound healing (e.g. burns, scleroderma), and cirrhosis [20]. In these situations, active fibroblasts grow abnormally but are not transformed, showing characteristic patterns of gene expression not observed in resting fibrocytes [19]. They also characteristically induce the formation of Fibroblast Activation Protein (FAP), a cell surface bound member of the serine protease gene family. The FAP antigen is an attractive antigenic target because it has broad applicability to several common and as yet poorly treatable cancer types, and it also displays a restricted expression in normal tissue, shown by detailed immunohistochemical analyses.

Molecular Signaling Pathways Implicated in Angiogenic Process

Numerous stimulators of capillary endothelial cell growth, termed angiogenic factors, have been identified since the search began 30 years ago. Traditionally, assignment of angiogenic activity to biomolecules was dependent on their ability to increase endothelial cell proliferation and/or to stimulate new capillary growth using in vivo assays, such as the chick Chorioallantoic Membrane (CAM) or the rodent cornea pocket (Box 1). Using these assays, various laboratories have described the purification of angiogenic factors not only from capillary rich sources, such as the brain and retina, but also from various untransformed and tumor derived cell lines. With the advent of reverse mouse genetics, we are now able to test the role of these candidates in vascular development and determine their functional requirements for the elaboration of a functional vascular tree. In addition, the careful analysis of mouse knockouts has revealed unforeseen roles for several matrix and signaling molecules in vascular growth and differentiation. As a result, it has become clear that no signaling pathway alone controls both vascular growth and organization. Rather, a number of different pathways cooperate to build, branch, and mature the growing vessel network. Below, we review findings on several key proteins implicated in the creation of new blood vessels during embryonic development and highlight their relevance to tumor biology. The factors that act as negative regulators of vascular development and their relevance to tumor angiogenesis are less well understood. We will therefore discuss this topic only briefly.
However, we will draw attention to the concept of endogenous angiogenesis inhibitors in the adult, because these factors, despite being nonessential for embryogenesis, can be specifically exploited in the clinic to block tumor angiogenesis.

**Vascular endothelial growth factor and its receptors**

The most potent and versatile angiogenic factor described to date is Vascular Endothelial Growth Factor (VEGF) (Figure 2). VEGF is a secreted, homodimeric glycoprotein with endothelial cell specific mitogenic activity and the ability to stimulate angiogenesis \textit{in vivo}. VEGF also stimulates vascular permeability, with an effect 50,000 times greater than that of the vasoactive substance histamine. In fact, the observation that tumor growth is associated with increased microvascular permeability provided the basis for VEGF’s original discovery and, accordingly, it was first named Vascular Permeability Factor (VPF). In addition to the founding member, the VEGF gene family now includes at least five new members that have been implicated in various aspects of cardiovascular growth and function.

VEGF and its tyrosine kinase-linked receptors, KDR/FLK1 and FLT1, are expressed in regions of blood vessel growth during embryogenesis. KDR/FLK1 is expressed in regions of the early mesoderm that are presumed to give rise to angioblasts, and it is the earliest known molecular marker for the endothelial cell lineage. During later stages of embryogenesis, FLT1 and KDR/FLK1 mRNA are both restricted to hematopoietic lineages and the endothelium of vascular cords and blood islands, with VEGF mRNA expressed in adjacent embryonic tissues. The mRNA levels for VEGF and its receptors decrease significantly postnatal, but expression is upregulated in the endothelium of tissues with ongoing angiogenesis, such as tumors, and is also elevated at sites proximal to fenestrated endothelium.

The observation that VEGF expression is regulated by hypoxia and glucose explains why VEGF levels are elevated in the ischemic regions of tumors and the retina and provides a molecular link between alterations in local tissue metabolism and growth factor control of angiogenesis.

Experimental data provide strong evidence for a necessary role of VEGF in developmental, adult physiological and pathological blood vessel growth. In the mouse embryo, targeted disruption of VEGF or its receptors leads to early embryonic lethality due to severe defects in vasculogenesis.

The postnatal blockade of VEGF function inhibits physiological neovascularization during bone growth and various aspects of the reproductive cycle. Consistent with a necessary role for VEGF in ischemia, neutralization of VEGF protein reduces the normal course of angiogenesis and disease severity in several experimental model systems. VEGF’s role as an angiogenic factor during diabetes, cancer, and chronic inflammation has made it a key target in the inhibition of angiogenesis and angiogenesis-dependent anthologies. In contrast, the major clinical problem of rescuing the viability of tissues starved of a proper blood supply due to obstruction or circulatory disorders demands clinical methodology for stimulating controlled blood vessel growth, for example, to alleviate coronary heart or diabetic disease in humans. Takeshita and Eisner colleagues first demonstrated the ability of VEGF to stimulate collateralization in experimental models of limb ischemia, and now major research efforts are focused on delivering VEGF protein, cDNA, or VEGF-expressing virus to human patients suffering from peripheral and coronary ischemia. A key feature of VEGF, which is becoming increasingly relevant to its use in human gene therapy, is that it is not a single protein but refers to a group of at least seven isoforms in humans; the predominant forms are secreted proteins of 121, 165, and 189 amino acids (VEGF121, VEGF165, and VEGF189) (Figure 2).
Box 1. Angiogenesis assays

The CAM assay

The chick Chorioallantoic Membrane (CAM) assay is perhaps the most widely used assay for Screening purposes, because it is a relatively simple and inexpensive method to identify angiogenic factors and is suitable for large-scale screening. Grafts of tissue and polymers or sponges soaked in putative angiogenic factors are placed on the CAM of developing chick embryos through a window in the eggshell. If an angiogenic factor is released, an increase of vessels is observed around the graft within 4 days after implantation. Blood vessels entering the graft can be visualized and counted under a stereomicroscope. Because young chick embryos lack a mature immune system, this assay is particularly useful to study angiogenesis elicited by tumor tissues. The major disadvantage of this assay is that the CAM contains a well-developed vascular network at the time of implantation, which can make it difficult to discriminate between new capillaries and existing ones.

The rabbit cornea pocket assay

The rabbit cornea is normally avascular. However, if sponges, polymers, or tissues containing angiogenic substances are implanted into pockets in the corneal stroma, vessels can grow out of the limbal region into the cornea. In albino rabbits, the vascular response can be readily quantified by stereomicroscopy and image analysis after perfusion of the cornea with Indian ink. This method is very reliable, but technically more demanding and more expensive than the CAM assay, and it is therefore not an ideal screening assay. Moreover, where suitable, the CAM assay may be preferred for ethical reasons.

Figure 2: Schematic illustration of the human VEGF-A isoforms. The VEGF isoforms are generated by alternative splicing from a single copy gene, termed VEGF-A. The isoforms differ by the absence or presence of domains that confer binding to heparin sulfate proteoglycans and neuropilin receptors and are encoded by exons 6 (6a or 6b) and 7. The VEGF183 isoform contains a deletion within exon 6a. The VEGF121 isoform does not contain any exon 6 or exon 7 domain. The murine orthologs of VEGF189, VEGF165, and VEGF145 and VEGF121 have been described; they are referred to as VEGF188, VEGF164, VEGF144 and VEGF120, because they are shorter by one amino acid.

These isoforms are generated by alternative mRNA splicing, which determines the presence or absence of heparin binding; the splicing pattern seems to be subject to complex temporal and spatial regulation during development. The biological significance of the VEGF isoforms is still under investigation. Two general ideas, by no means mutually exclusive, can be put forward to explain the function of the VEGF isoforms. The first model suggests that each isoform elicits a different signal within the responding endothelial cell, perhaps through isoform specific cell surface receptors. The interaction of VEGF165, but not VEGF121, with a cell surface receptor termed neuropilin (NP-1) supports this model. The presence of NP-1 amplifies KDR/FLK1-mediated chemotaxis and mitogenesis in cultured endothelial cells, either by directly increasing the affinity of VEGF165 for KDR/FLK1 or by enhancing receptor clustering. The second model suggests that differing affinities of the
isoforms for the heparin-rich matrix and cell surface results in their distinct localization within the extracellular milieu, thus providing a mechanism to regulate VEGF availability to target cells. While precise mechanistic details remain to be resolved, recent in vivo findings underscore the need to discern the relevance of VEGF isoform function to therapies aimed at pro- and antiangiogenesis in humans. Mice with targeted mutations that restrict VEGF expression to solely the 120 amino acid isoform, which lacks both NP-1 and heparin binding capacity, have recently been described. VEGF120 mice are born with a grossly normal vascular tree, but suffer from severe defects in postnatal blood vessel growth and symptoms of ischemic cardiomyopathy.

These observations suggest that VEGF isoforms exhibit a functional specificity that might be exploited in the design of proangiogenic therapies for ischemic cardiac and peripheral tissue disease. The expression of VEGF isoforms has been evaluated in various types of human cancer to determine their significance as prognostic factors for tumor growth and metastasis and to correlate isoform expression profiles with the properties of the tumor vasculature. However, a unifying theory of VEGF isoform function during tumor vascularization has not yet emerged.

In contrast, in mouse models of tumor angiogenesis differential VEGF isoform expression correlates with vascular network properties, as found in the embryo. In both situations, VEGF120 or VEGF188 alone do not support the formation of a robust vascular network. In contrast, VEGF164 alone is sufficient for the formation of a functional vasculature, presumably because of its ability to bind heparin while remaining partially mobile. However, it is the combination of soluble and heparin-binding VEGF isoforms that maximizes tumor growth, presumably because full vessel network functionality is achieved only when all isoforms cooperate. In addition to their diverse properties with respect to vascular patterning, the VEGF isoforms also differ in their potency to promote inflammation, as VEGF164 is more proinflammatory than the other isoforms. The heightened inflammatory properties of VEGF164 may contribute to the onset and progression of some forms of pathological neovascularization. However, the relevance of this observation to tumor angiogenesis has not yet been fully addressed.

**Fibroblast growth factors**

The Fibroblast Growth Factor (FGF) family of polypeptides was the first class of angiogenic factors described. Acidic FGF (aFGF or FGF1) and basic FGF (bFGF or FGF2) are potent mitogens and trophic factors for cultured endothelial cells, and both have potent angiogenic properties in the CAM and cornea assays (Box 1). The presence of FGFs, particularly bFGF, in tissues associated with new capillary growth made it an early favorite for a general angiogenic factor in vivo (Figure 3).

Molecular cloning and biochemical analysis has led to the unexpected discovery that both aFGF and bFGF lack signal sequence for secretion via exocytosis, therefore suggesting they normally reside inside the cell. Yet, in support of a role for these FGFs in extracellular signaling, a family of high-affinity tyrosine kinase cell surface receptors for FGFs has been described. This apparent contradiction might in part be explained by the finding that bFGF is released from cultured cells and animal tissues in vivo upon mechanical injury. These data have led investigators to suggest that the FGFs may exert angiogenic activities in situations associated with cellular damage, thus acting as a “wound hormone.” In situations where tissue injury does not play a role, the non-secreted FGFs may act as intracrine signaling factors that translocate to the nucleus to exert their activity. Consistent with this idea, both aFGF and bFGF possess a nuclear localization sequence and aFGF has been found in the nucleus of cultured vascular endothelial and smooth muscle cells.
Despite the fact that aFGF and bFGF are potent stimulators of angiogenesis in \textit{in vitro} models, the targeted disruption of both aFGF and bFGF does not appear to impair vascular development and results only in mild defects in hematopoiesis and wound healing. It therefore appears that, in contrast to VEGF, neither one of these FGFs is required for vascular growth.

![Figure 3](image)

\textbf{Figure 3}: The classes of secreted or cell-surface-associated molecules that cooperate to stimulate vascular growth and differentiation. The life cycle of endothelium may begin with the assembly of angioblasts into a primitive vascular plexus (vasculogenesis), or with the reactivation of quiescent vessel endothelium. Through the process of sprouting growth (angiogenesis), the vessel network begins to expand. New vessels acquire mural cells and adopt an arterial or venous identity; concomitantly, the vascular network continues to branch and remodel into a hierarchical vascular tree. Lastly, vessels specialize according to local physiological needs, for example to form the blood-brain barrier or the fenestrated sieve plates found in the kidney glomerulus. With the notable exception of blood-brain barrier function, most of the processes illustrated are stimulated by VEGF; several other factors act upstream of VEGF or cooperate with VEGF to affect specific aspects of vessel growth and specialization.

In support of the latter idea, FGFs can induce VEGF expression in the cornea assay and in cultured vascular smooth muscle cells and VEGF and FGFs synergize to promote neoangiogenesis \textit{in vitro} and \textit{in vivo}. Over the past decade, the FGF family has rapidly expanded in size and now includes 20 members, many of which are predicted to be secreted proteins. FGF3 and FGF4 can induce angiogenesis in the CAM assay and FGF7 induces neovascularization in the cornea assay (Box 1). FGF5 and FGF7 have also been implicated in angiogenesis. However, we will have to await the analysis of the relevant knockout mouse models to assess whether any of the more novel FGFs play an essential role in vascular growth and morphogenesis.

\textbf{Angiopoietins and the TIE2 Receptor Tyrosine Kinase}

The angiopoietins are ligands for the endothelial specific receptor tyrosine kinase TIE2 and are thought to act in a complementary and coordinated fashion with VEGF (Figure 3). Four angiopoietin ligands termed ANG1 to ANG4 are known, and together they provide the first example of a growth factor family in vertebrates that consists of both receptor activators and receptor blockers: while ANG1 and ANG4 act as TIE2 agonists, ANG2 and ANG3 behave as antagonists. Angiopoietins are likely to play a later role than VEGF in vascular development, since both ANG1 and TIE2 deficient mice form a normal primitive vascular plexus and therefore develop further than VEGF and KDR/FLK1 mutants. However, failure to stabilize and remodel the primary vascular plexus subsequently causes embryonic lethality of ANG1 deficient mice around the time of mid gestation. Ultrastructural studies suggest that these problems are due to a disruption of ANG1-mediated interactions between the endothelium and its supporting cells i.e., smooth muscle cells. In support of the idea
that ANG1 signaling is important for vessel remodeling, mutation of its receptor TIE2 is responsible for a heritable human disease characterized by thin-walled blood vessels with markedly reduced smooth muscle layers. Overexpression of ANG1 in the embryo results in hypervascularization, presumably by decreasing the normal amount of vessel regression that accompanies development. In contrast, overexpression of ANG2 results in embryonic lethality with phenotypes similar to those of the ANG1 and TIE2 knockouts, presumably because ANG2 blocks ANG1 activity by competing for TIE2. In support of the notion that angiopoietins also control the stability of mature vessels, ANG1 is widely expressed in adult tissues, while expression of ANG2 is present at sites of active physiological angiogenesis, such as the female reproductive tract and the placenta. Detailed analysis of ANG2 expression in the ovary has revealed that vessel growth and sprouting occurs at sites where ANG2 is co-expressed with VEGF. In contrast, expression of ANG2 in the absence of VEGF results in vessel regression. Taken together, these observations have led to the following model: ANG1 expression in the mesenchyme normally activates endothelial TIE2 to promote mural cell recruitment. Since ANG2 can compete for binding to TIE2, ANG1-dependent TIE2 activation is blocked in the presence of ANG2 and this leads to blood vessel destabilization. The exposed endothelium of destabilized vessels either degenerates, causing vessel regression, or, in the presence of VEGF, proliferates to yield net vessel growth. In support of the model of synergistic VEGF and angiopoietin signaling, their balance controls vascular permeability and tumor vascularization. It was shown that the initial vascularization of tumors is accompanied by high levels of ANG2, but these early vessels regress, causing necrosis in the tumor center. VEGF up regulation at the ischemic tumor margin then results in co-expression with ANG2 and a second wave of more stable vascularization is induced. Thus, sites of ANG2 expression correlate with vessel plasticity, and the outcome of vessel growth versus regression is decided by the presence of VEGF (Figure 3). Similar to ANG1, platelet-derived growth factor (PDGF-B) has been implicated in mural cell recruitment to blood vessels, but it remains to be elucidated how PDGF-B and angiopoietin signaling synergize to ensure mural cell investment of growing vessels (Figure 3).

**EPH/Ephrin signaling**

The transmembrane EPH receptors and their membrane-bound ephrin ligands comprise a signaling pathway with well-characterized functions during the development of several different organ systems, including the vasculature. EPHs and EPHRINs are often reciprocally expressed at tissue compartment boundaries and are best known for their roles in neural crest cell migration and axon guidance; however, it was attempts to better understand their role in neural patterning by Wang, Anderson and colleagues that revealed their role in vascular development. **EphrinB2** is widely expressed in the embryo, but within the vasculature it is restricted to arterial endothelium. Significantly, expression of its receptor **EphB4** predominates in the endothelium of veins. These findings provided the first molecular distinction between arteries and veins. Targeted inactivation of **EphB4** or **EphrinB2** leads to failure in the remodeling of the primitive vascular plexus and subsequent lethality at mid-gestation. Other EPH/EPHRIN family members are expressed in the vasculature, but expression is not always complementary between arteries and veins (e.g. ephrin A1 and B1 are expressed in both, and EphB3 is present in veins and the aortic arches). Instead, ligand/receptor expression is often reciprocal between blood vessels and their surrounding tissues, suggesting that paracrine signaling may occur between the endothelium and the mesenchyme. Based on the findings to date, it seems reasonable to suggest that members of the EPH/EPHRIN family are acting in the vasculature much in the same way as they do in the nervous system, where the complementary ligand/receptor expression patterns provide guidance cues by defining spatial boundaries in the developing embryo. A number of different EPHRINs and EPH receptors are overexpressed in a wide variety of human cancers. For
example, EPHRIN A1 and its receptor EPHA2 have been found on tumor vessels and tumor cells and may therefore contribute to both tumor-induced angiogenesis and tumor growth/spread, possibly by stimulating an autocrine loop. EPH/EPHRIN expression by tumor cells might also attract or organize their vascular supply, in analogy to the role of this signaling system during embryogenesis. In support of this idea, it has recently been shown that xenografted EphB4-expressing tumor cells can attract EPHRIN B2-positive blood vessels to increase their blood supply. However, it is presently not known whether dysregulated EPH/EPHRIN expression normally contributes to the vascularization of tumors or the chaotic organization of their vessel networks; further research will be necessary before we can conclude whether EPH/EPHRIN signaling might provide a valid target for antiangiogenic therapy in cancer.

**NOTCH signaling**

Like the EPH/EPHRIN system, the NOTCH signaling pathway has been studied extensively in embryos, where it regulates the specification of cell fate through local cell interactions. The NOTCH proteins are transmembrane receptors that are activated by membrane-spanning ligands of the DELTA, SERRATE and JAGGED families. Both NOTCH receptors and ligands are expressed in specific vascular compartments, that is, arterial or venous endothelium or vascular smooth muscle cells/pericytes. During vascular patterning, VEGF may act upstream of NOTCH to promote arterialization, but it also promotes other aspects of angiogenesis: loss of Notch4 and/or Notch1 impairs vascular morphogenesis in the embryo, and loss of function for Notch3 or Jagged 1 causes hereditary vascular diseases in humans. The contribution of NOTCH signaling to tumor angiogenesis is not yet clear. However, recent genetic studies in the mouse and human provide compelling evidence for a role of NOTCH signaling in the adult vasculature, raising the possibility that this pathway may present a target for tumor angiogenesis. For example, DELTA-4 is an endothelial-specific NOTCH ligand that is upregulated in tumor vasculature.

**Hypoxia and oxidative stress**

**Hypoxia:** Hypoxia and oxidative stress often occur concurrently within tumours and both act as strong angiogenic stimulators. Hypoxia is a decline in oxygen below the normal levels found in a tissue (5%), manifesting itself in acute and chronic vascular disease, pulmonary disease, and cancer. During neoplasia intratumoral hypoxia is associated with a poor clinical outcome, and resistance to conventional treatment modalities such as radiotherapy. This is primarily because neoplastic cells adapt to hypoxic environments and subsequently survive and even proliferate under these conditions, resulting in aggressive tumour behavior [23]. Growing tumours develop hypoxic regions because the new blood vessels form an aberrant vascular network, have poor blood flow, and the stores of oxygen and glucose are rapidly metabolized, so that the tumour effectively outgrows its blood supply [24]. The irregularity of these vessels and their inability to provide oxygen and nutrients to the surrounding tissue stimulates the release of growth factors, which induce tumour angiogenesis and contribute to the malignant phenotype. Endothelial cells are critical in the maintenance of vascular homeostasis and are directly affected by hypoxia, because they must specifically cope with the variations in oxygen tension that occur in the blood. The vascular endothelium undergoes molecular modifications in response to reduced oxygen levels, which impact significantly on EC function, and may result in changes to cell–cell interactions [25]. Exposure to hypoxia activates ECs in one of two ways. Acute hypoxia promotes the recruitment, adherence, and activation of neutrophils to blood vessels by the release of inflammatory mediators and growth factors. While in chronic hypoxia there is elevated expression of specific genes, which encode various cytokines and growth factors involved in angiogenesis, including PDGF and VEGF [23].
As tumours outgrow angiogenesis or are deprived of oxygen, a gene expression response to hypoxia is initiated. These genes regulate several biological, including cell proliferation, apoptosis, and angiogenesis. Although several transcription factor pathways are involved in hypoxia regulation, most attention has focused on Hypoxia Inducible Factors (HIFs).

These are heterodimeric transcription elements of which HIF-1 is the best described. HIF-1 comprises the HIF-1α and HIF-1β sub-units with HIF-1α being the key transcription factor. HIF-1 regulates DNA response elements, in turn controlling the expression of more than 40 target genes [26]. In response to low cellular oxygen levels, HIF-1 binds to hypoxia response elements, which activate the expression of numerous hypoxia response genes such as VEGF, PDGF, endothelin, Insulin-Like Growth Factor-II (IGF-II), adrenomedullin, and Endothelial Growth Factor (EGF), which have pro-angiogenic effects [27]. The intrinsic role that HIF plays in angiogenesis has been examined using both in vitro and in vivo studies, with investigators showing that loss of HIF-1 activity impacts negatively on tumour growth and vascularization [26]. Analyses using embryonic stem cells lacking the HIF-1α gene showed reduced levels of VEGF in vitro and in vivo tumour xenografts derived from this cell line had fewer vessels and significantly impaired vessel function [28]. In addition, HIF-1α knockout mice display inefficient angiogenesis and are characterized by a complete lack of cecal vascularization [29]. In the last two years, the pathways mediating the hypoxia signal have been elucidated; HIF-1α is post-translationally modified on two prolyl residues by prolyl hydroxylases that require oxygen as a cofactor as well as ferrous iron, vitamin C and 2-oxoglutarate [30]. There are three well-characterized enzymes that have prolyl hydroxylase domains (PHDs 1, 2, and 3) [31]. PHD-2 is hypoxia inducible and the most widely expressed. The consequence of hydroxylation is that HIF-1α is then recognized by the Von Hippel Lindau protein, which is an ubiquitin ligase that targets HIF-1α for destruction in the proteasome [32]. Clearly as oxygen becomes less available, there is less modification and therefore HIF-1α is stabilized.

**Oxidative stress:** Reactive Oxygen Species (ROS) naturally occur in many biological systems and are prevented from damaging molecules such as DNA, proteins, and lipids by the cell’s antioxidant defenses, which supposedly scavenge free radicals and subsequently prevent DNA damage. During oxidative stress, the balance between ROS and antioxidants is disrupted in favor of ROS, which subsequently become toxic to the cell. It is unclear if this is due to excessive ROS production or loss of antioxidant defenses [33]. It has been established that oxidative stress induces mutations in tumor suppressor genes and causes DNA damage, including base modifications, frame-shift mutations, and DNA strand breaks, critical molecular events in the initial stages of neoplasia [33]. An imbalance in ROS also stimulates tumor cells to release stress-induced angiogenic factors such as VEGF, IL-8, and the Matrix Metalloproteinase-1 (MMP-1). Release of these molecules also occurs in the presence of Thymidine Phosphorylase (TP), a stimulator of angiogenesis and oxidative stress in tumor lesions, whose expression correlates with poor clinical outcome [34,35]. Increased TP activity promotes angiogenesis in a range of pathologies and TP over-expression correlates with altered vascular density and poor prognosis in many human tumor types. Despite the wealth of data linking TP and angiogenesis, the molecular mechanisms underlying this link remain unclear. Site-directed mutagenesis and antibody studies have proved that promotion of vessel growth by TP is dependent on its enzyme activity which catabolizes thymidine to thymine and 2-dideoxyribose-1-phosphates. This reaction induces carcinoma cell oxidative stress, causing tumor cells to secrete stress-induced angiogenic factors (VEGF, IL-8, and MMP-1), which promote angiogenesis [34,35].

**Antiangiogenic Therapies**

Anti-VEGF-therapies can lead to regression of already existing tumor vascularization.
VEGF is essential for tumor vessel cells to survive; it protects them from apoptosis and promotes tumor growth without a continuing supply of VEGF, endothelial cell apoptosis occurs, and newly developed tumor microvessels decay. VEGF inhibition also can lead to both structural and functional changes on surviving vessels, a phenomenon described as vessel normalization [36].

**Bevacizumab (Avastin™)**

Bevacizumab (Avastin™) is a recombinant monoclonal antibody directed against VEGF. Bevacizumab binds to VEGF and inhibits VEGF receptor. A precursor antibody to Bevacizumab was A4.6.1, a murine antibody cloned by Ferrara (Leung et al. 1989) and bound with high affinity to different isoforms of VEGF. It inhibited cell growth in immortalized tumor cell lines by a significant reduction of vascular. As a murine protein, it provoked anaphylactic reactions and needed to be humanized. In preclinical studies, the combination of Bevacizumab with chemotherapy led to synergistic activity. In xenotransplants, the combination of Bevacizumab with capecitabine inhibited tumor growth more effectively and longer than any other tested substance (Sachsenmaier 2001). It also showed synergistic effects in combination with paclitaxel and Trastuzumab (Herceptin™), a humanized monoclonal antibody that acts on the HER2/neu (erbB2) receptor. In further invivo studies, the application of Bevacizumab to animals previously treated with capecitabine, topotecan, or cisplatin showed more successful tumor suppression. Also, repeated application of Bevacizumab proved to be safe and well tolerated.

**Cetuximab (Erbitux™)**

Cetuximab (Erbitux™), a monoclonal antibody, binds to the extracellular domain of EGFR, competing with its specific ligands and inhibiting intracellular signaling. Further, as an IgG1 immunoglobulin, it could elicit host antitumor immune responses such as cell-mediated antibody dependent cytotoxicity and also EGFR internalization, down-regulation, and finally receptor degradation. Among the EGFR targeting substances, Cetuximab has been approved for combination with radiotherapy for the treatment of locally advanced Squamous-Cell Carcinoma of the Head and Neck (SCCHN) or as a single agent in patients who have had prior platinum-based therapy. Side effects of Cetuximab treatments include acne-like skin affections, fever, and chills, asthenia, and nausea.

**Small Molecule Tyrosine Kinase Inhibitors**

Small molecule Tyrosine Kinase Inhibitors (TKI), such as sorafenib (Nexavar™) and sunitinib (Sutent™), also represent antiangiogenic agents. Sorafenib is a potent orally available protein kinase inhibitor. Originally identified as a Raf kinase inhibitor, Sorafenib also inhibits VEGFR-1 and 2, Platelet-Derived-Growth Factor Receptor (PDGFR-β), and c-Kit-Protein. Sorafenib has a dual antitumoral target affecting the tumor cell and its blood vessels. In human endothelial cells and in smooth muscle cells, VEGFR-2 signaling and activation of Extracellular Signal-Regulated Kinase (ERK) are induced. Sunitinib (Sutent™) is also an orally available multitargeted TKI. Especially in renal cell carcinoma and in Gastrointestinal Stroma Tumors (GIST), sunitinib proved to be superior to earlier therapy strategies and is now established as first-line treatment. Sorafenib and sunitinib are both approved for the treatment of renal cell carcinoma. A clinical phase III trial studying sunitinib compared to sorafenib or placebo in treating patients with kidney cancer that has been removed by surgery (ClinTrails.gov NCT00326898) is currently recruiting patients.

**Cediranib (Recentin™)**

Cediranib (Recentin™), known as AZD2171, is an oral, highly potent, inhibitor of VEGF signaling that selectively inhibits all known VEGFR tyrosine kinase activity (VEGFR-1, -2
and -3) (Figure 4). Encouraging results obtained to date with Cediranib in a range of clinical studies show its potential as a new antiangiogenic drug in combination with radiotherapy. The ability of Cediranib to inhibit growth factor stimulated receptor phosphorylation was determined in a range of cell lines [37]. Furthermore, this effect was also associated with inhibition of MAP kinase phosphorylation, a downstream marker of VEGF signaling. These data suggest that Cediranib can selectively inhibit VEGFR-dependent proliferation, but appreciable functional selectivity is evident versus other targets, including EGFR, FGFR, and PDGFR-α. The \textit{in vivo} activity of Cediranib was also investigated in a model of vascular sprouting. In nude mice implanted with a VEGF-containing Matrigel plug, Cediranib completely abolished VEGF-induced vessel formation [37]. Furthermore, Cediranib has demonstrated antitumor efficacy in a number of \textit{in vivo} preclinical studies, including xenograft, orthotopic, metastatic, and spontaneous models of human cancer [37]. Administration of Cediranib produced dose dependent inhibition of tumor growth in a range of histologically distinct human tumor xenografts (lung, colon, breast, prostate, and ovarian) and also decreased primary tumor growth, metastasis, and microvessel density in an orthotopic model of murine renal cell carcinoma [38]. Taken together, Cediranib has shown anti-tumor activity in a range of preclinical \textit{in vivo} models consistent with inhibition of VEGF signaling and an antiangiogenic mode of action rather than a direct antiproliferative effect on tumor cells. In an extensive phase I program, Cediranib was tested as monotherapy in prostate cancer, with carboplatin and paclitaxel in Non-small Cell Lung Cancer (NSCLC), with selected chemotherapy regimens in advanced cancer, and with gefitinib in advanced cancer. Cediranib is one of the most potent inhibitors of VEGFR-2 tyrosine kinase activity in development. Preclinical studies have demonstrated that Cediranib inhibits VEGF dependent signaling, angiogenesis, and neovascular survival. Cediranib is also a potent inhibitor of VEGFR-1 and -3 tyrosine kinases, and shows selectivity for VEGFRs versus a range of other kinases. Consistent with an antiangiogenic effect, once-daily treatment with Cediranib produced dose-dependent inhibition of tumor growth in a broad range of established human tumor xenografts. A series of phase I studies have been conducted to investigate Cediranib in patients with cancer, both as monotherapy and in combination with certain other anticancer strategies. These investigations have shown Cediranib to be generally well tolerated, with a side effect profile that is tolerable and manageable. Currently available pharmacokinetic data are supportive of a oncedaily oral dosing schedule for Cediranib. Furthermore, preliminary efficacy data demonstrate that Cediranib has potential antitumor activity in multiple tumor types. Recruitment to a number of clinical trials has been initiated to further determine the activity of Cediranib in a wide range of tumors. Currently ongoing trials address the effect of Cediranib on metastatic colorectal cancer in combination with different chemotherapies. Encouraging preliminary results were reported for Cediranib in patients with glioblastoma suggesting an increase in overall survival [39].

![Figure 4: Intracellular signaling inhibitions by Cediranib.](image)
Conclusion

Angiogenesis research is clearly at an exciting time. The next few years will show whether the first antiangiogenics such as VEGF blockers have sustained clinical efficacy. Blood vessel growth has turned out to be complex, with many factors playing a role in the process. This complexity in itself, however, leads to many opportunities for therapeutic targeting that will test the ingenuity of cancer biologists and clinicians over the next decade.

References


Abstract

Mutations which are changes in DNA sequence are one of the most important causes of cancer which is one of the most important issues of our time. A lot of types of cancers especially colorectal cancer occurs as a result of errors occurring in genes. In order to protect of our genetic integrity namely elimination of errors in DNA sequences, various mechanisms are working. Checkpoints in cell cycle and DNA repair mechanism are the important mechanisms for mutation prevention.

Introduction

Alterations in DNA are called mutations. Mutations may result from mistakes made during DNA replication. In addition, chemicals or physical forces can damage a segment of DNA before it is replicated. Unless these errors are detected and corrected before the next replication, they may be passed on to future cells or could even prevent the DNA from being copied at all. In other words, the DNA needs to be in as good a shape as possible before it is replicated.

When the DNA of a somatic cell (a cell of the body other than a gamete) is severely damaged, it may not be possible to repair all the errors. In this case, substantial changes in cell function and even life-threatening cancers may result. For example, skin cancer can be caused by damage to DNA from excessive exposure to sunlight. Mutations of somatic cells, fortunately, are not passed to the offspring.

On the other hand, mutations that occur in the gametes (sperm or egg) may be passed to future generations. These are the changes that lead to evolutionary change, including changes that cause species to diverge. Generally speaking, these heritable mutations occur very slowly. The DNA of humans and chimpanzees, for example, differs by only about 1% even though our evolutionary paths diverged over 5 million years ago. Two humans differ by perhaps one base pair in a thousand, but that is still 3 million base pair differences, more than enough to account for all of our individual variation [1].

Mutations can be either spontaneous or induced. Spontaneous mutations are rare and result from errors in DNA replication. Induced mutations are caused by environmental factors, such
as radiation, chemical substances, smoking, etc. The frequency of these mutations depends on the type and duration of the agent (mutagen) and for some it can be very high [2].

Singular mutation may cause spontaneous cancer. Endogenous causes of mutation include depurination and depyrimidation of DNA; proofreading and mismatch errors during DNA replication; deamination of 5-methylcytosine to produce C to T base pair substitutions; and damage to DNA and its replication imposed by products of metabolism (notably oxidative damage caused by oxygen free radicals). Deficiencies in cellular defense mechanisms may also provoke spontaneous mutation. These include defective DNA excision-repair; low levels of antioxidants, antioxidant enzymes, and nucleophiles that trap DNA-reactive electrophiles; and enzymes that conjugate nucleophiles with DNA-damaging electrophiles. Mechanisms underlying many of those cellular defenses are under genetic control. Thus, germ line mutations or polymorphisms of genes that govern them may also contribute to spontaneous cancer [3].

A mutagen is a physical or chemical compound that changes the DNA and thus increases the frequency of mutations above the natural background level. As many mutations cause cancer, mutagens are typically also carcinogens. The nature of mutagens varies; they are usually chemical compounds (e.g., ethidium bromide, sodium azide) or ionizing radiation (e.g., UV, gamma, alpha radiation). Mutagens can get inserted into the DNA strand during replication, react with DNA, and cause structural changes, or work indirectly by causing the cells to synthesize chemicals that have a direct mutagenic effect. By exerting their modification, carcinogens and mutagens have the potential to activate oncogenes or inactivate tumor suppressor genes, thus contributing to the neoplastic process [4].

The radiation may ionize and damage a DNA molecule directly, but since tissue consist of about 80 percent water, most of the ionizations occur in water molecules and lead within less than a microsecond to the production of highly reactive H\(^+\) and OH\(^-\) free radicals. These, in turn, can produce major damage in DNA. In any event, the end result may be a mutation that can lead to the formation of a cancerous cell [5].

Chemical mutagens are categorized into four general groups, based on the mechanism by which they interact with DNA

1. Base analogues are structurally similar to bases; they have their mutagenic effect by being incorporated into DNA and causing mispairing during replication.

2. Intercalating agents are generally flat molecules that can fit between bases, producing helix distortions that can lead to replication errors.

3. DNA-reacting chemicals, such as reactive oxygen, can directly modify bases, changing coding groups and thereby allowing base pairing with the wrong base.

4. Alkylating agents bond covalently to DNA and result in the addition of some organic group to the bases or possibly to the sugar-phosphate backbone [6].

The extent of a mutation can range from a change to a single base pair (point mutation) to the alteration of a large region of a chromosome (chromosome aberration). Mutations can occur anywhere in the total DNA of an organism. In humans, approximately 95% of DNA does not code for any gene products. As a result, many mutations have no effect on the phenotype, because they are located in regions of the genome that have no impact on cellular functions. By contrast, a mutation within an exon of a structural gene can alter the functioning of the gene product and cause a dramatic phenotypic change [7].
There are many mechanisms that can raise mutation rates. Chromosomal instability increases genomic rearrangements, defective DNA repair raises the rate of point mutations, and processes that control methylation can alter the rate of change in methylation patterns and gene regulation. It would be interesting to compare changes in these mutational mechanisms during periods when lineages face new environmental challenges and during periods of relative environmental stability [8].

A tumor develops through repeated rounds of mutation and proliferation giving rise eventually to a clone of fully malignant cancer cells. At each step, a single cell undergoes a mutation that either enhances cell proliferation or decreases cell death so that its progeny become the dominant clone in the tumor. Proliferation of each clone hastens the occurrence of the next step of tumor progression by increasing the size of the cell population at risk of undergoing an additional mutation. The final step depicted here is invasion through the basement membrane, an initial step in metastasis. In reality there are more than the three steps shown here and a combination of genetic and epigenetic changes are involved [9]. It has been suggested that as many as seven distinct genetic changes are required for a cell to progress from adenoma to carcinoma, and that the accumulation rather than the specific nature and temporal order of the mutations is most critical [10].

Cancer cells display a broad spectrum of genetic alterations that include gene rearrangements, point mutations, and gene amplifications, leading to disturbances in molecular pathways regulating cell growth, survival, and metastasis. When such changes manifest in majority of patients with a specific type of tumour, these can be used as biomarkers for detection and developing targeted therapies, besides predicting responses to various treatments [11-13].

**Mutational Basis of Carcinogenesis**

Under normal circumstances, two classes of regulatory genes control the cell’s activities: proto-oncogenes and tumor suppressor genes. Mutated or damaged proto-oncogenes that contribute to cancer are called oncogenes. Some oncogenes drive the internal rate of cell growth and division faster than normal. Others produce damaged protein receptors that fail to heed inhibitory growth signals from other cells. However, it is important to recognize that one oncogene alone is not sufficient to cause cancer. Because so many cellular processes must be disrupted at once and each process may be controlled by multiple genes, cancer develops only when multiple oncogenes are present [14].

Proto-oncogenes encode proteins that are involved in the control of cell growth. Alteration of the structure and/or expression of proto-oncogenes can activate them to become oncogenes capable of inducing in susceptible cells the neoplastic phenotype. Oncogenes can be classified into five groups based on the functional and biochemical properties of protein products of their normal counterparts (proto-oncogenes). These groups are (1) growth factors, (2) growth factor receptors, (3) signal transducers, (4) transcription factors, and (5) others, including programmed cell death regulators [15].

Of the many known oncogenes, all but a few are derived from normal cellular genes (i.e., proto-oncogenes) whose products participate in cellular growth-controlling pathways. Conversion, or activation, of a proto-oncogene into an oncogene generally involves a gain-of-function mutation. At least three mechanisms can produce oncogenes from the corresponding proto-oncogenes. The mechanisms are as follows point mutations in a proto-oncogene that result in a constitutively acting protein product, localized reduplication (gene amplification) of a DNA segment that includes a proto-oncogene, leading to overexpression of the encoded protein, chromosomal translocation that brings a growth-regulatory gene
under the control of a different promoter and that causes inappropriate expression of the
gene. An oncogene formed by the first mechanism encodes an oncoprotein that differs
slightly from the normal protein encoded by the corresponding proto-oncogene. In contrast,
the latter two mechanisms generate oncogenes whose protein products are identical with
the normal proteins; their oncogenic effect is due to their being expressed at higher-than-
normal levels or in cells where they normally are not expressed. However they arise, the
gain-of-function mutations that convert proto-oncogenes to oncogenes act dominantly; that
is, mutation in only one of the two alleles is sufficient for induction of cancer [16].

In normal cells, c-myc links growth factor stimulation and cellular proliferation [17-19].
MYC is a potent oncogene that can promote tumorigenesis in a wide range of tissues [20-26]. MYC is the most frequently amplified oncogene and the elevated expression of its gene
product, the transcription factor c-myc, correlates with tumor aggression and poor clinical
outcome [27,28]. Elevated expression of c-myc occurs through multiple mechanisms in
tumor cells, including gene amplification, chromosomal translocation, single nucleotide
polymorphism in regulatory regions, mutation of upstream signaling pathways, and
mutations that enhance the stability of the protein [18,19,29,30].

Some of the most frequently mutated genes in human tumors are those of the ras gene
family. These genes are mutated in more than 40 percent of human tumors. The ras gene
family encodes signal transduction molecules that are associated with the cell membrane
and regulate cell growth and division. Ras proteins normally transmit signals from the cell
membrane to the nucleus, stimulating the cell to divide in response to external growth
factors. Ras proteins cycle between an inactive and active state by binding either GDP or
GTP. When a cell encounters a growth factor, growth factor receptors on the cell membrane
bind to the growth factor, resulting in autophosphorylation of the cytoplasmic portion of the
growth factor receptor. This causes recruitment of proteins known as nucleotide exchange
factors cause ras to release GDP and bind GTP, thereby activating ras. The active, GTP-
bound form of ras then sends its signals through cascades of protein phosphorylation in
the cytoplasm. The end point of these cascades is activation of nuclear transcription factors
that stimulate expression of genes whose products drive the cell from quiescence into the
cell cycle. Once ras has sent its signals to the nucleus, it hydrolyzes GTP to GDP and
becomes inactive. Mutations that convert the proto-oncogene raw to an oncogene prevent
the ras protein from hydrolyzing GTP to GDP and hence freeze the ras protein into its on
conformation, constantly stimulating the cell to divide [31].

Many tumor suppressors have activity in both normal and tumor cells; whereas the
others, such as p53, are inactive in normal cells and only activated by potential cancer risks.
A tumor suppressor may possess multiple mechanisms to suppress cancer cell growth [32].
For example, the most important tumor suppressor p53, which is associated with about 50%
of human cancer cases [33], can trigger DNA repair processes, induce the transcription
of other tumor suppressors, such as p21 and p16, and initiate cell apoptosis [32,34,35].
Despite the tremendous growth in cancer research and identification of numerous tumor
suppressors [36], the exact underlying mechanisms through which the tumor suppressors
function are not always clearly revealed. To date, four major mechanisms have been revealed
for tumor suppressors: suppression of cell division, induction of apoptosis, DNA damage
repair and inhibition of metastasis [37].

Tumor-suppressor genes generally encode proteins that in one way or another inhibit
cell proliferation. Loss of one or more of these “brakes” contributes to the development of
many cancers. Five broad classes of proteins are generally recognized as being encoded by
tumor-suppressor genes: Intracellular proteins, such as the p16 cyclin-kinase inhibitor, that
regulate or inhibit progression through a specific stage of the cell cycle, receptors for secreted hormones (e.g., tumor derived growth factor β that function to inhibit cell proliferation, checkpoint-control proteins that arrest the cell cycle if DNA is damaged or chromosomes are abnormal, proteins that promote apoptosis and enzymes that participate in DNA repair. Although DNA-repair enzymes do not directly function to inhibit cell proliferation, cells that have lost the ability to repair errors, gaps, or broken ends in DNA accumulate mutations in many genes, including those that are critical in controlling cell growth and proliferation. Thus loss-of-function mutations in the genes encoding DNA-repair enzymes promote inactivation of other tumor-suppressor genes as well as activation of oncogenes [16].

The Retinoblastoma Tumor Suppressor Protein (Rb) functions to regulate multiple critical cellular activities, including the late G1 restriction point, the DNA damage response checkpoint, cell cycle exit, and differentiation [38-41]. However, the Rb gene is infrequently mutated or deleted, instead upstream pathways that regulate Rb by phosphorylation on Cdk sites are altered in the majority of human cancers, including deletion and mutation of the p16 tumor suppressor and upregulation and mutation of cyclin D1, D2, D3, Cdk4 and Cdk6 genes [42,43,38,40-44].

**Mutational Basis of Colorectal Carcinogenesis**

The morphologic changes from normal mucosa to early, intermediate, and late adenoma and finally to colorectal carcinoma are associated with certain essential mutations that occur in sequence [45]. Based on genetic analysis, at least two pathways are characterized in detail, which lead to colon cancer development. One pathway (indicated with red arrows) initiates with mutations in the Adenomatous Polyposis Gene (APC-gene) and Chromosomal Instability (CIN) followed by mutations in K-ras, Deleted in Colorectal Cancer (DCC) and p53 genes [46]. Patients with germline mutations in the tumor suppressor gene adenomatous polyposis coli (APC-gene) are prone to develop familial adenomatous polyposis. The APC-gene product is involved in cellular adhesion and intercellular communication, and both functions are absent after gene mutations. Mutations in the APC-gene can frequently be found in adenomas, including small adenomas, suggesting that they are an early event [45]. Central lesions in both hereditary and sporadic colon tumors result in activation of the Wnt signaling pathway. In nearly all tumors, deactivating APC or GSK3β mutations or stabilizing CTNNB1 (encoding β-catenin) mutations are present [47]. The normal function of β-catenin is to bind the Transcription Factors TCF (Transcription factor) and LEF (Lymphoid Enhancer-Binding Factor). The β-catenin/TCF/LEF complex subsequently activates gene transcription. With accumulated β-catenin in the APC deficient cell, transcriptional targets are excessively activated [48]. More specifically, the canonical tumor suppressor function of APC is to form a “destruction complex” with Axin/Axin2 and GSK-3β (Glycogen Synthase Kinase) that promotes the ubiquitination and subsequent proteasomal degradation of the oncogene β-catenin in the absence of Wnt signaling. Loss of APC function results in an accumulation of β-catenin, which translocates to the nucleus and engages the TCF/LEF transcription factor complex to activate transcription of a large number of target genes including cyclinD1, c-myc, and CRD- BP [49]. The tumorigenic consequences of unregulated β-catenin activity may be related to both the direct stimulation of cellular growth and proliferation, and to the disruption of differentiation programs [50]. The second important mutation may occur in the K ras oncogene, a cell membrane-bound signal-transduction molecule. Oncogenes are positive regulators of growth-promoting signaling pathways. Mutations in K-ras resulting in continuous activation of ras occur in about half of colorectal cancers and adenomas larger than 1 cm but are found less frequently in small adenomas. Thus, K-ras mutations are early events but occur later than APC-gene mutations. Late adenomas and colorectal cancer frequently show loss of the tumor suppressor gene DCC
DCC deletion is, however, uncommon in early adenomas, placing this event after the K-ras mutations. The DCC gene product is a cell surface molecule involved in adhesion. Finally, deletion of the tumor suppressor gene p53 is observed in 75% of colorectal cancers but is infrequently observed in adenomas and is therefore a late event in carcinogenesis. The mutations and deletions of the APC, K-ras, DCC, and p53 genes are the most commonly detected genetic alterations in colon cancer (Fearon and Vogelstein, 1990). The second pathway (indicated with blue arrows) is initiated by the Mutations In The Mismatch Repair (MMR) genes and Microsatellite Instability (MSI) followed by mutations in hMSH3, hMSH6, TGFβIIIR, IGFIIR, PTEN, BLM, TCF-4, Bax and E2F4 genes. Other pathways are less characterized, but a high degree of overlapping is expected among them. At least, seven gene mutations are needed to develop a normal epithelial cell into carcinoma. However, a cluster of gene and chromosome aberrations such as p15, p16, Bub1, cyclin D1, tPa, CEA, Nm23, MMP, E-Cadherin (CDH1), CD44, 7q, 14q, 22q and p are observed in carcinoma and metastatic tumors. ASEF, APC-stimulated guanine nucleotide exchange factor; DLG, Drosophila discs large; EB1, end-binding protein 1, KAP3A, kinesin superfamily-associated protein 3A; MCR, mutator cluster region; NES, nuclear export signal; NLS, nuclear localization signal; PP2-B56α, protein phosphates 2A B56α subunit.

**Mechanisms of Mutation Prevention**

DNA damage is a relatively common event in the life of a cell and may lead to mutation, cancer, and cellular or organismic death. Damage to DNA induces several cellular responses that enable the cell either to eliminate or cope with the damage or to activate a programmed cell death process, presumably to eliminate cells with potentially catastrophic mutations. These DNA damage response reactions include: (a) removal of DNA damage and restoration of the continuity of the DNA duplex; (b) activation of a DNA damage checkpoint, which arrests cell cycle progression so as to allow for repair and prevention of the transmission of damaged or incompletely replicated chromosomes; (c) transcriptional response, which causes changes in the transcription profile that may be beneficial to the cell; and (d) apoptosis, which eliminates heavily damaged or seriously deregulated cells. DNA repair mechanisms include direct repair, base excision repair, nucleotide excision repair, double-strand break repair, and cross-link repair [51] (Figure 1).

**Checkpoints**

Multiple pathways are involved in the maintainance of genetic integrity, most of which link to the cell cycle [52]. The inactivation of these pathways as part of a multi-step process contributes significantly to the origin of tumors. By arresting the cell cycle, checkpoints presumably allow cells to repair DNA. Checkpoints can be seen as a network of surveillance systems, i.e., signal transduction systems that interrupt cell cycle progression, when damage to the genome or failure of a previous activity in the cell cycle is detected [52-53].

The cycle cannot proceed without a series of successive events occurring. If an event fails, then the cell arrests at defined checkpoints to allow adjustments to be made. The most notable checkpoints occur at the G1-S and G2-M transition points. The G1-S checkpoint allows the cell to repair any DNA damage before it is copied in the S-phase, to prevent mutations becoming fixed in the genetic material. The second, G2-M checkpoint allows the cell to ensure that the chromosomes are arranged correctly prior to segregation to the daughter cells [54].

In response to DNA damage, checkpoints arrest the cell cycle in order to provide time for DNA repair. DNA damage checkpoints are positioned before the cell enters S phase (G1-S checkpoint) or after DNA replication (G2-M checkpoint) and there appears to be DNA damage checkpoints during S and M [56].
An important part of the cellular response to DNA damage is checkpoint activation—checkpoint kinases CHK1 and CHK2 phosphorylate key proteins to elicit cell-cycle blocks. Inhibiting these kinases was believed to sensitize tumour cells to cancer treatments that damage DNA, because in the absence of checkpoints and efficient DNA repair, the response would switch to cell death or senescence [57].

In order to signal cell cycle arrest, for example after DNA damage, checkpoint control pathways must sense the damage and then transduce the signal. To delay cell cycle progression after DNA damage, these mechanisms affect the activity of critical cell cycle regulators. The integrity of these checkpoints is therefore considered pivotal in maintaining genetic stability. Mutations in the checkpoint components may lead to aberrant cell cycle progression in the presence of perturbing stimuli, including DNA damage, and subsequently to genetic instability [58].

At the G1/S checkpoint, cell cycle arrest induced by DNA damage is p53-dependent. Usually, the cellular level of p53 is low but DNA damage can lead to rapid induction of p53 activity [59]. p53 stimulates the transcription of different genes including p21, Mdm2 and Bax [60]. The induction of p21, a CKI, results in CDK inhibition and cell cycle arrest, preventing the replication of damaged DNA [61]. Mdm2 plays an important role in the regulation of p53: it binds to and inhibits p53 transcriptional activity and contributes to the proteolytic degradation of p53 by facilitating its ubiquitination, hereby providing a negative feedback loop [62]. Binding of regulatory proteins can also modulate p53 ubiquitination: the p19 (ARF) protein, encoded by the ARF-INK4 locus (see below), binds to Mdm2 and this prevents the Mdm2-mediated p53 proteolysis [63]. In the case of severely damaged cells, p53 induces cell death by activating genes (e.g. Bax, Fas and genes involved in oxidative stress pathways) that are involved in apoptotic signalling [64-66]. Different protein kinases ‘recognize’ DNA
damage, e.g. Ataxia-Telangiectasia-Mutated (ATM), Ataxia and Rad3 Related (ATR). These kinases phosphorylate p53 in response to DNA damage, resulting in p21 blocking the cell cycle, at least at the G1/S checkpoint [67]. DNA Protein Kinase (DNA-PK), a DNA double-strand break repair enzyme is related to ATM and ATR, but it is not known yet whether it also plays an important role at the G1/S checkpoint. [68-69].

This checkpoint prevents the improper segregation of chromosomes, which is likely to be important in human tumorigenesis [52,70].

The G2/M checkpoint plays an especially important role in ensuring the propagation of error-free copies of the genome to each daughter cell [71].

The G2/M checkpoint pathways converge mainly on activity and intracellular localization of the CDK1/cyclin B1 complex. Activation of this complex is essential for cells to enter mitosis. Just prior to mitosis, CDK1/cyclin B1 complexes translocate to the nucleus and trigger the initiation of mitotic changes like chromosome condensation and nuclear membrane breakdown [72].

Control of Cyclin B-CDK1 activity may take place at two levels; a direct control of Cyclin B-CDK1 phosphorylation status by WEE1, MYT1, and CDC25, as well as a more indirect regulation via the AURORA A/BORA/PLK1 network [73].

The critical transition from metaphase to anaphase and the separation of sister chromatids is monitored by the spindle checkpoint gene products that include the Mad (mitotic arrest defective) proteins, Mad1-3p, the Bub (budding uninhibited by benomyl) proteins, Bub1-3p, and Mps1 [74]. To progress through this transition, cells must proteolytically degrade a number of proteins that are required earlier for entry into mitosis and this is accomplished by the activation of the proteasome, a component of the large multiprotein complex referred to as the Anaphase-Promoting Complex or APC [75-77].

**Repair**

Defects in DNA repair give rise to hypersensitivity to DNA-damaging agents, accumulation of mutations in the genome and finally to the development of cancer and various metabolic disorders. The importance of DNA repair is illustrated by DNA repair deficiency and genomic instability syndromes, which are characterized by increased cancer incidence and multiple metabolic alterations. Up to 130 genes have been identified in humans that are associated with DNA repair [78].

DNA repair pathways play an important role in maintaining the integrity of the genome, and it is obvious that defects in repair pathways are involved in many different types of diseases, including leukemia and cancer [79].

An updated inventory of about 150 human DNA repair genes is described. The compilation includes genes encoding DNA repair enzymes, some genes associated with cellular responses to DNA damage, and other genes associated with genetic instability or sensitivity to DNA damaging agents [80].

Base Excision Repair (BER) protects the genome from chemical and physical threats from the environment. Apparently, absence of each of the DNA glycosylases separately results in small or moderate changes in spontaneous mutation frequencies, and no obvious immediate effects at the individual level. It is likely that DNA glycosylases have a major role in protecting the long-term integrity of the genome [81].

A specific N-glycosylase that recognizes a particular damaged base initiates BER. N-glycosylase binds to the altered nucleotide and cleaves the N-glycosylic bond between
the base and the sugar, producing an Apurinic/Apyrimidinic (AP) or abasic site. The AP site is processed by an AP endonuclease or glycosylase-associated AP-lyase, in which the phosphate backbone adjacent to the AP site is cleaved to generate a base gap, a single-strand nick, or nucleotide overhang(s) when more than one nucleotide is involved, at the lesion. DNA polymerase inserts the correct base(s), and DNA ligase seals the nick, thereby repairing the damage [82].

Nucleotide Excision Repair (NER) can be described in six interconnected steps: (1) initial damage detection in which the lesion is first marked by a protein, (2) damage verification in which a second protein or protein complex authenticates the presence of a damaged nucleotide, (3) Dual-strand incision in which the phosphate backbone is hydrolyzed in two places on the same strand several nucleotides away from the damaged site, (4) excision of the lesion and surrounding nucleotides, (5) repair synthesis in which replication of one strand is performed to fill in the gap left by the removal of the oligonucleotide containing the damage, and (6) DNA ligation in which the newly synthesized repair patch is sealed [83].

The mechanism of Homologous Recombination (HR) repairs chromosomes that harbour DNA Double-Strand Breaks (DSBs) and other types of damage. In mitotic cells, the repair of DSBs by HR most often involves the use of the intact sister chromatid as an information donor, and therefore occurs primarily in the late S and G₂ phases of the cell cycle, when the sister chromatid becomes available. Because the sister chromatid is identical in sequence to the damaged DNA molecule, the repair reaction faithfully restores the genetic configuration of the injured chromosome and is viewed as being error free [84,85]. The replication of a damaged DNA template, such as one that harbours a DNA nick, can lead to a broken DNA replication fork. The newly formed sister chromatid serves to direct the repair of the damaged DNA so as to prevent fork demise [86]. hRAD51, hRAD52, hRAD54, XRCC2, XRCC3, Brca2 are involved in HR pathway [87].

The first step in HR is resection of the DSB in a 5′-3′ manner that involves the human Mre11-Rad50-Nbs1 (MRN) complex and CtIP [88-89]. The resultant 3′ single strands are bound by Replication Protein A (RPA), preventing degradation and providing the signal to activate the cell cycle checkpoint kinase ATR [90]. Next, hRad51 is recruited and assembled into a nucleoprotein filament, displacing RPA. This reaction involves several accessory proteins including hRad52, XRCC2, XRCC3 and BRCA2 [91-93]. The recruitment and assembly of hRad51 nucleoprotein filaments can be visualized in cell nuclei as foci formed after IR and in S phase at sites of replication-associated DSBs [94]. Strand invasion by the hRad51 nucleoprotein filament into the adjacent homologous sister chromatid results in formation of a D loop structure. DNA synthesis from the invading 3′ end extends the D loop, increasing its stability [95,96]. The extension of the D loop also permits capture of the second DNA end, resulting in the formation of a Holliday junction. Resolution of the Holliday junction by a resolvase, such as Gen1 [97], completes the repair, generating two identical sister chromatids [98]. Alternatively, in break-induced replication, the entire sister chromatid is copied following formation of the D loop structure [99]. Exchange of DNA strands between homologous DNA molecules via recombination ensures accurate genome duplication and preservation of genome integrity [100].

DSB repair can also occur by a different mechanism known as non-homologous DNA end-joining, which is much more error prone than HR [84,85]. DNA-PKcs, Ku70, Ku80, DNA ligase IV, XRCC4 are involved in non-homologous DNA end-joining pathway [87]. The formation of a two-ended DSB, for example, by ionizing radiation, is indicated. The DNA ends are substrates for binding of the Ku70/80 heterodimer, which localizes DNA-PKcs to the ends and promotes their juxtaposition. If no further processing of the ends is required,
the additional core components of non-homologous DNA end-joining, XRCC4, DNA ligase IV, and XLF can complete the rejoining reaction. Alternatively, end processing may require the activities of the nuclease Artemis and/or the DNA polymerases TdT, pol lambda, and pol mu. The Ku heterodimer likely plays a central role in orchestrating the activities of the proteins involved in non-homologous DNA end-joining. Transient reversible interaction of the processing factors with the core components provides great flexibility in the combination of broken ends that can be rejoined because this does not require a strict order in which the processing factors engage or in which the four strands will be processed [101].

**Conclusion**

Cells have been exposed to many types of environmental stresses, and these stresses can sometimes lead to sub-lethal damage. In order to survive and function under adverse conditions, it is necessary to repair or eliminate DNA damage, and as a consequence, cells have developed a number of complex repair systems to enable their survival and functioning. Knowledge and understanding of these complex systems will make contributions to biology and medicine [102].

Rapid developments in the field of medical genetics associated with oncogenic properties. Therefore for prevention of cancer, control of cell growth, analysis of cell cycle, checkpoints and cell pathways, assessment of DNA damage and restoration requires evaluated together beyond the single gene disorders.

In this chapter; the effects of mutational basis of carcinogenesis, mutational basis of colorectal carcinogenesis, mechanisms of mutation prevention, checkpoints and repair on cancer prevention were discussed.

**References**


The hypothesis that the immune system plays a role in the antitumor response and can be manipulated for the treatment of cancer was advanced as early as the 1890s, when William Coley used bacterial extracts to treat patients with sarcoma. It was thought that the ensuing inflammatory response successfully induced regression of large tumors by activating the immune system and inducing immune cells to attack the tumor. Since then, nonspecific forms of immunotherapy have been used with varying degrees of success for the treatment of a limited number of malignancies—(i) BCG adjuvant for superficial bladder cancer and high-dose IL-2 for metastatic melanoma [1], and (ii) donor lymphocyte infusions for leukemic relapse after allogeneic stem cell transplant [2]. However, these strategies were often accompanied by serious and potentially life-threatening toxicities. Advances in immunology, in the understanding of the requirements for T-cell activation and tolerance, and the development of novel technologies to analyze and augment immune response, now provide tumor immunologists with the opportunity to translate more broadly applicable principles in immunology to practice of treating patients with cancer in a more specific and effective manner. This chapter on tumor immunology begins with a description of the endogenous immune response, followed by a discussion of the components involved, [including T cells, Dendritic Cells (DCs)], and finally a summary of immunotherapeutic strategies arising from an understanding of the antitumor immune response.

Immune System and Cancer

The ability of the immune system to effectively respond to tumors is dependent on the following assumptions:

a) Tumor cells differ from normal cells.

b) The immune system can recognize these differences.

c) The immune system is in an active state and capable in generating an effective and protective immune response. These prerequisites indicate that cancer immune editing is
a dynamic process that involves both the tumor as well as the immunocompetent effector system. The efficient eradication of tumors in a living organism requires crosstalk between leukocytes of the innate and adaptive arms of the immune system, which reside in different immunological compartments. It has been shown that the cytokine interferon-gamma (IFN-γ), and the cytolytic effector molecules perforin and granzyme are secreted by cells of the innate and adaptive immune system, which contribute to the host’s immune defense against cancer. Following uptake into tumor cells, intracellular located granzyme B initiates apoptosis via the activation of procaspases 3, 7, 10 inactive cytosolic inhibitor of Caspase-Activated Dnase (ICAD), and the disruption of the membrane potential of mitochondria, which causes the release of cytochrome c into the cytosol. The situation of the host’s immune defense is complicated by the fact that throughout evolution, tumors have adopted strategies to interfere with and to overcome the immune system. These immune escape mechanisms involve the downregulation of Major Histocompatibility Complex class I (MHC I) and costimulatory molecules, the loss of tumor-specific antigens, the stimulation of inhibitory receptors expressed on effector cells, the stimulation of the growth of inhibitory CD4/CD25 double-positive regulatory T cells (Tregs), and the secretion of inhibitory molecules such as serpin- protease inhibitors, which interfere with the apoptosis cascade. For example, about 60% of metastases express significantly reduced levels of MHC class I on their cell surfaces. These findings indicate that a better understanding of the interaction between immune cells, tumor cells, and the tumor microenvironment and their consequences will guide the development of more effective approaches for controlling and successfully treating cancer.

**Immune surveillance**

In early attempts to demonstrate immunity to tumours, tumours were transplanted from one animal to another. The transplants were rejected and this was taken as evidence of immunity to the tumour. Later it was recognized that these experiments demonstrated transplantation immunity directed against MHC antigens. When genetically homogenous inbred animals (mice) became available it became possible to investigate tumour immunity and it was shown that if an animal was immunized with a tumour and was challenged with a graft of the same tumour, the graft was rejected. A graft of a different tumour was not, showing that the animal was specifically immune to the immunizing tumour. At about the same time Burnett (1973) [3] and later Thomas (1982) [4] put forward the theory of immune surveillance against tumours. They proposed that tumours arose frequently and that the majority are eliminated by the immune system. Burnett summarized his view of immune surveillance as follows:

(1) Most malignant cells have antigenic qualities distinct from those of the cell type from which they derive, (2) such antigenic differences can be recognized by T cells and provoke an immune response. If this view is correct it follows that, (3) the incidence of malignant disease should be greatest in periods of relative immunological inefficiency, particularly in the perinatal period and old age, (4) immunosuppression whether genetic or induced by drugs, radiation, infection, or other causes should increase the incidence of cancer, (5) spontaneous regression of tumours may occur and evidence of an immune response should be apparent in these cases, and (6) large-scale histological examination of common sites of cancer should reveal a higher proportion of tumours than become clinically apparent. Burnett also suggested ways in which the theory of surveillance might be tested experimentally. Thus, immunosuppressive agents should facilitate the transfer of tumours, or damage to the T cell immune response produced by surgical removal of the thymus might lead to increased tumor incidence.

At first sight, a variety of clinical and experimental data do seem to be in accord with the
surveillance theory. In man, some tumours show a higher incidence in the first few years of life than in early adulthood and the incidence, but of different tumour types, and then rise progressively with increasing age. The incidence of tumours is also greatly increased in immunosuppressed individuals who are treated with immunosuppressive drugs to prevent rejection of the grafted kidney [5]. However, a closer examination does not support the surveillance hypothesis. The age incidence of tumours is as well explained by many other theories of cancer causation as by immunosurveillance. Tumours are caused by genetic changes in their cells of origin. These changes might be expected to occur either as errors during periods of rapid cell division (early life) or when external causes (carcinogens) have had time to take effect as in later life. The data derived from immunosuppressed individuals are similarly less straightforward to interpret when examined more closely. Although there does seem to be a slight increase in the frequency of most tumours, there is a disproportionate increase in a few tumour types (Table 1). The relative risk of suffering from some rare tumour types may be increased more than 1000-fold in immunosuppressed compared to normal individuals. An experiment in mice provided a possible explanation for these results.

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Relative risk</th>
<th>Virus involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaposi’s sarcoma</td>
<td>&gt;1000</td>
<td>HHV8</td>
</tr>
<tr>
<td>Lymphoma Of the brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>&gt;1000</td>
<td>EBV</td>
</tr>
<tr>
<td>7.4</td>
<td></td>
<td>EBV</td>
</tr>
<tr>
<td>EBV</td>
<td>320</td>
<td>Papillomaviruses</td>
</tr>
<tr>
<td>Skin carcinoma</td>
<td>40</td>
<td>Papillomaviruses</td>
</tr>
<tr>
<td>Liver carcinoma</td>
<td>30</td>
<td>Hepatitis B</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>6.4</td>
<td>HTLV 1</td>
</tr>
<tr>
<td>Cervix carcinoma</td>
<td>4.2</td>
<td>Papillomaviruses</td>
</tr>
<tr>
<td>Digestive system carcinoma</td>
<td>2.6</td>
<td>------</td>
</tr>
<tr>
<td>Respiratory system carcinoma</td>
<td>2.1</td>
<td>------</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>1.1</td>
<td>------</td>
</tr>
</tbody>
</table>

Table 1: Immunosuppression and tumours.

When a large group of mice were treated from birth with anti-lymphocyte serum (raised in rabbits), their T cell immunity was greatly depressed. The mice did not develop large numbers of spontaneous tumours but when they inadvertently became infected with polyomavirus a number of them developed multiple tumours of a type characteristically caused by this virus [6]. Similarly, the lymphoid tumours seen in transplant recipients (Table 1) contain DNA and proteins of the Epstein–Barr virus (EBV), a herpes virus implicated in the cause of Burkitt’s lymphoma (a B-cell tumor seen in parts of Africa) and nasopharyngeal carcinoma. The virus can also immortalize normal human B lymphocytes in vitro. These findings suggest, therefore, that the most important role of the immune system in tumor protection may be in preventing the spread of potentially oncogenic viruses. This view agrees with experimental and clinical data on EBV, an ubiquitous infectious agent in human populations. Following infection the virus is carried lifelong and the individual also has lifelong immunity. Under normal circumstances, immune CD8 cytotoxic T lymphocytes can be demonstrated in vitro and there is thus a balance between virus production and the immune response, while in immunosuppressed individuals the immune system is unable to prevent virus spread.

The T cells of such individuals cannot kill EBV-transformed B cells in vitro, and virus can often be isolated from body tissues and secretions such as saliva. Several other viruses have now been implicated in causing human tumours and the risk of acquiring these tumours is generally increased in immunosuppressed patients (Table 2). Although worldwide virally induced tumours are a major cause of cancer, since hepatitis B virus and papillomaviruses
infect millions of individuals, there are also many cancers where no viral involvement can be detected. If it is accepted that immune surveillance operates principally against oncogenic viruses, what is the role of the immune system in relation to other tumors? Evidence from experimental animals (suggests that there are immune responses to many tumors and the slight increase of relative risk for tumors with no known viral involvement would support this (Table 1). However, the fact that most tumors grow and kill the host, suggests that the immune response is probably a late event and in most cases is unable to prevent tumor outgrowth. Nevertheless, that there is an immune response suggests that tumors do contain antigens recognized as foreign by the immune system. If this is the case it should be possible to boost immunity to them by deliberate immunization.

Although the relative risks of Kaposi’s sarcoma and brain lymphoma are very high, the majority of immunosuppressed patients do not get these tumors since they are very rare in non-suppressed individuals. In contrast, most transplant patients eventually acquire skin tumors since, although the relative risk is lower, these are much commoner tumors in normal individuals. HTLV 1 causes adult T-cell leukaemia only.

<table>
<thead>
<tr>
<th></th>
<th>Immat-ure DC</th>
<th>Mature DC</th>
<th>Activation signals for DC maturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td></td>
<td>Increased &quot;veil&quot; and dendrite appearance</td>
<td>Bacterial products:</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>+++</td>
<td>-</td>
<td>LPS (lipopolysaccharide)</td>
</tr>
<tr>
<td>Costimulatory molecules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DR (*MHC CLASS II)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD40</td>
<td>+/</td>
<td>+++</td>
<td>Teichoic acid</td>
</tr>
<tr>
<td>CD80 (B7-1)</td>
<td></td>
<td></td>
<td>CpG DNA</td>
</tr>
<tr>
<td>CD83</td>
<td></td>
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<td>Viral products: dsRNA</td>
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<tr>
<td>CD86(B7-2)</td>
<td></td>
<td></td>
<td>CD40 ligand</td>
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<tr>
<td>Chemokine-receptors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCR7</td>
<td>-</td>
<td>++</td>
<td>TNFα, PGE2</td>
</tr>
<tr>
<td>CCR2</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>CCR6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Immature versus mature DCs – surface and functional phenotypic differences.

**Effector Cells in Tumor Immunity**

Effectors of adaptive immunity can be ascribed to a “humoral” and a “cellular” arm represented, respectively, by B cells that mediate effects through production of antibodies, and T cells that interact directly with target cells through the T-Cell Receptor (TCR). In humoral immunity, antibodies binding surface proteins on tumor cells can kill via complement activation or by bridging targets with cytotoxic cells through a process known as ADCC (Antibody-Dependent Cell-Mediated Cytotoxicity). In this process, the Fc portion of antibody couples with receptors on macrophages or NK cells that then effect cell killing. Although antibodies are highly effective in vitro, convincing evidence that antibody responses elicited in vivo play a critical role in antitumor immunity is weak. However, the significance of humoral responses with respect to tumor immunity has been supported by the identification of serum antibodies to potentially immunogenic tumor antigens and the successful therapy of patients using monoclonal antibodies.

**Dendritic cells**

Dendritic cells are specialized or “professional” APCs that are activated during the innate
immune response and are uniquely equipped to take up and present antigen to effector cells of the adaptive immune system—the antigen specific CD4 and CD8 T cells. DCs are so-named because of pseudopods or “dendrites” which are processes that extend from the cell to facilitate antigen presentation. In vivo, the induction of an antitumor immune response may occur by tumor cells presenting antigen directly to T cells, but it is believed that a more common and robust pathway for tumor-specific T-cell activation in vivo is by cross-presentation. This is a process by which antigens released by necrotic, dying, or apoptotic tumors are taken up by DCs and represented to T cells under more favorable stimulatory conditions in the tumor-draining lymph node.

Dendritic cells can be characterized in their immature or mature forms based on contrasting surface and functional phenotype (Table 2). In their immature form, DCs are well equipped to capture antigens through surface receptors such as the C-type lectins (e.g. DEC-205, mannose receptors), αβ integrins, or CD36 for internal processing and presentation. DC activation via “danger signals” mediated through some of these abovementioned receptors and other surface receptors (e.g. the TLRs), leads to DC maturation [7] in the presence of bacterial or viral products, TNF-α, or prostaglandins. It is also thought that in addition to DC activation by these receptors, cooperation of CD4 helper T cells is required to “license” DCs with the capacity to stimulate CD8 T cells through interaction of the CD40 ligand on CD4 T cells with CD40 on DCs. Upon maturation, further antigen uptake by DCs is downregulated, and, in preparation for optimizing T-cell activation, DCs upregulate surface expression of the T-cell costimulatory molecules (CD80, CD83, and CD86), and secrete cytokines such as IL-7 and IL-12 that facilitate full T-cell activation. In the case of tumor immunity, DCs circulate through the blood and accumulate at tissue sites in response to chemokines arising from the site of tumor necrosis or inflammation. As immature DCs, antigen is collected and processed for presentation on the surface in the context of MHC molecules. Following maturation and upregulation of surface costimulatory molecules, lymphokines, and the chemokine receptor CCR7, DCs traffic to lymph Nodes where T-cell activation can occur.

**T cells**

**Antigen presentation and T-cell stimulation:** In contrast to B cells that provide “humoral” immunity through the production of soluble antibodies, T cells mediate “cellular” immunity by interacting directly with their target cells. T cells achieve specificity for cells expressing the target antigen through the surface TCR that recognizes fragments of antigen (usually peptide fragments) presented by the Major Histocompatibility Complex (MHC) through either a Class I or Class II processing pathway (Figure 1).

Since proper antigen processing and presentation by the MHC is critical to antigen-specific immunity, a brief description of the MHC complex is presented.

The MHC is encoded by highly polymorphic genes that cluster on chromosome 6 in humans and are codominantly expressed. Human MHC Molecules are called Human Leukocyte Antigens (HLAs) and are divided into Class I and Class II HLA or MHC that present peptides to CD8 and CD4 T cells, respectively. For the most part, Class I MHC molecules are represented in humans by HLA-A, -B, and -C family of alleles, and Class II MHC by HLA-DR, -DQ, and –DP families. The Class I MHC complex comprises three parts: the MHC-encoded heavy α chain, a non-MHC-encoded β2-microglobulin chain, and a 8-11-mer peptide sitting in a groove formed by the polymorphic region of the α chain. The Class II MHC complex comprises three parts, the polymorphic α and β chains and a 10-30+ mer peptide sitting in a groove formed by the two chains. These parts are assembled in cytosolic compartments together with peptides derived from tumor proteins that have undergone
proteasome-mediated cleavage to peptides of the appropriate length. The peptide–MHC complex is then transported to the surface where the immunogenic peptide sitting in the MHC groove is presented to the TCR. The T cell recognizes the peptide only in the context of the MHC complex; therefore, mutations affecting any component of the antigen-presenting machinery can abrogate specific T-cell recognition and killing of tumor cells.

**Box 1: T-cell-APC interaction**

Tumor-derived peptides are processed within the APC and presented by the peptide MHC complex to their cognate TCR. For CD4 T cells, optimal T-cell activation requires the ligation of costimulatory molecules such as CD28 with B7. Upregulation of CD40 ligand following TCR engagement delivers a “licensing” signal to APC that result in increased B7 expression and production of T-cell modulatory cytokines such as IL-12. T-cell activation is downregulated by the inducible inhibitory receptor, CTLA-4, which blocks CD28 mediated signals and competes for B7 binding. For CD8 T cells, activation following TCR engagement with the Class I MHC complex may be enhanced through costimulatory signals delivered by 4-1BB and other counter receptors. IL-2 and other cytokines produced by activated CD4 T cells provide growth signals to cognate CD8 T cells.
**Costimulatory and inhibitory T-cell signals:** In addition to the interaction of the TCR with the peptide-MHC complex, the activation of T cells can be modulated by the engagement of surface costimulatory or accessory molecules by their respective ligands on antigen-presenting cells (see Box 1). The most prominent of these are the signals provided by CD28 upon binding to B7-1 (CD80) or B7-2 (CD86) on APC. B7–CD28 interaction mediates signals that can fully activate an antigen-driven T-cell response, enhance T-cell survival by up-regulation of anti-apoptotic Proteins such as BCL-x L and drive proliferation. Absence of B7 has been associated with T-cell anergy while engineered expression of B7 in potentially immunogenic tumor cells can induce tumor rejection in murine models. While B7-CD28 interaction appears to be critical to the generation or priming of an effective antitumor response, it does not influence the effector or killing phase of T cells. Hence, T cells generated with a B7-transduced tumor vaccine can eradicate B7-negative tumor. Other costimulatory molecules that deliver a positive signal to T cells include ICOS (inducible costimulator), OX40, 4-1BB, and other B7 family members (e.g. B7-H3). Accessory/adhesion molecules, such as ICAM-1 and LFA-1, are also critical to T-cell recognition. These molecules converge in and reinforce the TCR-peptide-MHC synapse, by forming in aggregate with other molecules, a supra-molecular activation complex to facilitate delivery of a longer-lasting, more potent T-cell signal. Cytotoxic T Lymphocyte Associated Antigen-4 (CTLA-4) delivers a negative regulatory signal to activated T cells and competes with CD28 for binding to B7 on target cells(see Box 1). CTLA-4 is an inducible receptor with a greater affinity for B7 than CD28; however, in contrast to CD28, its surface expression is non-constitutive and relatively short lived. CTLA-4 is believed to provide an immunologic “brake” to prevent overly robust and potentially damaging overstimulation by suppressing T-cell proliferation through IL-2 inhibition and down-regulation of cell cycle activity. CTLA-4-deficient mice develop splenomegaly and a lympho-proliferative pathology. Since many tumor target antigens are also normal self proteins, eliminating CTLA-4 inhibition may provide a means of breaking tolerance to self-antigens and augment an otherwise muted T-cell response to tumor. Administration of anti-CTLA-4 antibody in some murine Models results in organ autoimmunity but can also lead to rejection of previously non-immunogenic tumors. In clinical trials, administration of anti-CTLA-4 antibody has produced signs of autoimmune toxicity as well as tumor regression in individuals receiving a tumor-specific vaccine.

**T lymphocytes:** T cells can generally be divided into helper CD4 T cells and cytotoxic or killer CD8 T cells. Helper CD4 T cells recognize antigen in the context of MHC Class II and can be further differentiated into Th1 and Th2 subsets on the basis of distinct cytokine and receptor profiles. Th1 CD4 T cells produce IL-2 and interferon-γ, express IL-12 and IL-18 receptors, and regulate T-cell immunity, while Th2 T cells produce IL-14, IL-15, and IL-13, and regulate B cell immunity. It is believed that a Th1- type response would be beneficial in antitumor immunity since it mobilizes a T-cell-mediated response. Cytotoxic CD8 T cells recognize antigen in the context of MHC Class I and, when activated, release perforin and toxic granules that mediate direct cell killing by punching holes in the cell membrane to facilitate entry of enzymatic packets (granzymes A and B). Although most studies have weighed in on a greater role for the cytotoxic CD8 T lymphocyte (CTL) in tumor eradication, the helper CD4 T lymphocyte has also been shown to be a vital component in the induction and maintenance of a competent antitumor immune response. Not only have tumor antigen-specific responses been identified for CD4 T cells but the presence of CD4 T cells may be required for CTL responsiveness. Acting in concert, both CD4 and CD8 T cells provide for synergistic mechanisms of tumor killing. CD8 T cells kill tumor cells through the release of perforin and granzymes A and B, or through engagement of the death receptor, Fas, through Fas Ligand (FasL) expressed on activated T cells. FasL – Fas interaction leads to a form of cell death known as apoptosis. In contrast to necrosis or death due to cell injury, apoptosis
or programmed cell death involves a stepwise cascade of events initiated by receptor engagement at the cell surface (in this case, Fas), leading to DNA fragmentation. CD4 T cells can kill tumor cells directly by FasL – Fas engagement, as well as through indirect mechanisms that involve the recruitment of nonspecific effectors, such as macrophages and eosinophils, that can act even on MHC-negative tumors (Figure 2).

**Figure 2: Effector mechanisms of T cells.** Activated CD8 T cells deliver a "death" signal to tumor cells through FasL – Fas interaction 1. CD8 T cells may also kill tumor cells directly through perforin and granzymes released upon engagement of the TCR2. Perforin exocytosed in CTL granules form spores in the tumor cell membrane. Granzymes enter tumor cells through pores and induce tumor cell death 3. CD4 T cells can mediate tumor death through Fas interaction. Activated CD4 T cells may also mediate cytotoxicity indirectly through the release of interferon-γ and IL-5 to recruit tumoricidal macrophages (m φ) and eosinophils (Eos) 4.

**NK cells:** Natural Killer cells are activated during the innate response by the inflammatory milieu that is established by invading tumor cells. These effector cells are not antigen specific and do not express a TCR but do kill tumor through Killer Activating Receptors (KARs) expressed on their surface. Engagement of KARs with tumor derived ligands, such as MICA and MICB, which are upregulated in infected or “stressed” cells, such as tumor cells, leads to NK cell activation and tumor cell death. NK cells also engage self- MHC Class I molecules on target cells through inhibitory receptors (Killer Inhibitory Receptors – KIRs), perhaps, as a means of preventing auto reactivity. The loss of MHC expression on tumor cells, a process that can develop during carcinogenesis and immune-selection lends itself to preferential NK cell activation. The contribution of NK cells to the endogenous antitumor response in vivo may be best exemplified in a thymic nude mouse that have no T cells, but retain a population of functional NK cells that appears to be sufficient to mediate tumor resistance. In humans, NK-type cells can be expanded in vitro with high doses of IL-2 for adoptive transfer; however, in this setting, their efficacy isles s well defined and treatment is often accompanied by serious toxicity. The in vivo augmentation of NK-type cells may also be one mechanism by which high-dose IL-2 therapy has shown some clinical effect in the treatment of patients with metastatic melanoma or renal cancer.

**Regulatory T cells:** A population of T cells with regulatory properties that control autoimmune and antitumor responses was postulated as early as 1975; however, convincing evidence for their existence has been elusive. Recently, a population of CD4+, CD25+ T cells that possess immunosuppressive function has been identified. This discovery has led to a renewed understanding of the role of regulatory cells. CD4+ regulatory T cells (Tregs) are represented by two subsets – naturally occurring T regs representing 5-10% of peripheral T cells and induced Tregs that develop from conventional CD4+ CD25- T cells. Naturally occurring T regs mediate their suppressive properties through cell-to-cell contact by an unknown mechanism. Although activation is dependent on TCR engagement, their
suppressive effects are nonspecific. They are known to express Glucocorticoid-Induced TNF Receptor (GITR) and CTLA-4, a known T-cell inhibitor of T-cell costimulation. However, the role of CTLA-4 and GITR in mediating the suppressive effects of naturally occurring Tregs is not well defined. Induced or adaptive Tregs can be generated from conventional CD4+ CD25 T cells following in vitro exposure to antigen and IL-10 and the induced Treg cells themselves appear to mediate their inhibitory properties through the production of IL-10 and TGF-β. Tregs have been found to be fundamental for the control of autoimmune responses in several murine models, such as inflammatory bowel disease, and depletion of CD25+ T cells has been shown to mediate immune rejection of various murine tumors in vivo, presumably through the release of suppressive effects on T cells targeting shared self-tumor antigens [8]. Elevated frequencies of CD4 Treg cells have also been described in cancer patients, leading to the design of clinical trials involving the administration of anti-CD25 antibodies to augment an endogenous antitumor immune response.

**Immunotherapy: From Preclinical Limitations into Actions**

**Problems facing immunotherapy**

**Introduction:** Immunotherapy is treatment by immunological means. In active immunotherapy the tumour bearer’s own immune system is stimulated to respond to the tumour while in passive immunotherapy, immune cells or their products are given. Immunotherapy may also be specific or nonspecific. Specific active immunotherapy aims to stimulate only a response to the tumour or deliver passively agents such as monoclonal antibodies that target the tumour. Non-specific therapy boosts all immune responses, for example, through the use of cytokines or Lymphokine Activated Killer (LAK) cells. This section will deal mainly with the principles underlying and problems facing, attempts to use active immunization. Experimental evidence suggests that CD4 and CD8 T cells and antibody may all play a role in effective immunotherapy in different animal tumour models. However, generally T cells appear to play a major role in immunity to solid tumours, while antibodies are more effective against leukaemia or lymphoma. Most active immunization has therefore sought to induce strong cellular immune responses, which have been shown to be capable of eliminating large tumour masses in experimental animals and humans [9,10]. An additional reason for doing so is that the target antigens need not be cell surface molecules, since processed peptide epitopes reach the cell surface to be recognized by T cells (Figure 3).

**Induction of anti-tumour immune responses:** antigens and adjuvants: For immunization against tumours, what antigen to use is the first problem to be faced. Although increasing numbers of tumour antigens are being defined by SEREX (recombinant tumor cDNA expression libraries), using T cell clones or by sequencing peptides eluted from tumour MHC antigens, individual tumours vary in antigen expression. This means that for any tumour type it would be sensible if possible, to immunize against several antigens (as is the case for most vaccines against microorganisms). As yet this is rarely possible in humans so that in practice most human experiments have taken one of two approaches. Either immunization is with antigens known to be well expressed on most tumours of a particular type, including CEA for colon cancer, PEM in breast cancer, or MAGE in melanoma. Alternatively, whole tumour cells are used. Irrespective of the antigen used, the aims of immunization will be to induce a strong CD4 and CD8 -cell response against the chosen antigen(s). One problem that particularly applies to immunization against tumours rather than microorganisms is the possibility that damaging responses to self-antigens might be induced. As discussed above, most tumour-associated molecules are unaltered self-molecules, often expressed, though usually at a lower level, in normal tissues as well as tumours. That this is a real problem is shown by experiments in which mice undergoing successful immunotherapy against a melanoma became de-pigmented [11] and patients
have exhibited vitiligo (de-pigmentation) while undergoing anti-melanoma immunotherapy [12]. This particular side effect is not life-threatening but autoimmune responses to other antigens might be. Selection of antigens as target for immunotherapy should therefore take into account the tissue distribution of the antigens. It is a disadvantage of the use of whole tumour cells as antigen that it is impossible to control which antigens the host responds to. The recognition that ‘danger signals’ were essential for initiation of immune responses has provided an explanation for why an immune response might be a late event in the evolution of a tumour. This and recognition that tumour cells lacked co-stimuli such as CD80 or CD40 led to experiments in which tumour cells were transduced with genes coding for costimuli or cytokines (effectively internal ‘danger signals’).

Figure 3: Interactions in an immune response. Antigen is taken up by DCs, which migrate to the draining lymph node. Soluble antigen also reaches the node in the afferent lymph. In the node, DCs present antigen to CD4 and CD8 T cells and B cells encounter soluble antigen. Clonal expansion of T and B cells takes place and Cytotoxic T Lymphocyte (CTL) and T-helper effector and memory cells are generated. B cells develop into Plasma Cells (PCs). Effector cells leave the node and migrate via lymph and blood to the original site of antigenic stimulation.

However, evidence that immune responses to tumours are induced following uptake of tumour antigens by APC, suggests that optimal strategies for induction of antitumour responses should target tumour antigens to APCs. This has led to immunotherapy based on the use of antigen-loaded and activated DCs [13]. For this it is necessary that the DCs are MHC-matched with the patient, in practice usually autologous, and obtaining sufficient DCs is laborious, technically demanding, and expensive. Alternative means of targeting DC in vivo are being explored. Interestingly, giving antigen plus Granulocyte Monocyte Colony Stimulating Factor (GM-CSF), a cytokine that is chemo attractant for DCs, has been shown to be an effective means of immunization [14]. In effect, such a strategy mimics the release of cytokines, induced at a site of inflammation by ‘danger signals’, that leads to accumulation of inflammatory k cells. A similar effect can be achieved by the use of adjuvants. These are substances that potentiate immune responses in several ways. They provide ‘danger signals’, often delivered by incorporated bacterial products, they often provide a slow release depot of antigen and their physicochemical properties, for example, particulate materials, may promote entry of antigen into the cytosol and endogenous antigen processing. At present very few adjuvants are licensed for human use and the most well-established, aluminium salts, favour Th2 responses. However, new adjuvants are becoming available [15] and new immunization strategies are being developed to induce strong and long-lasting cellular (CD4
and CD8) responses. To date, the so-called prime/boost regimes appear to be one of the most effective [16]. In this method the antigen is first presented in one form, often as DNA, and the subjects are boosted with antigen presented in a different form, often in recombinant vaccine or adenoviruses. These induce inflammation (‘danger signals’) and a large secondary immune response is induced and effector cells produced. Whatever the target antigen and means of immunization are, it is important that both CD4 and CD8 responses are induced concurrently, as recent evidence has shown that CD8 memory cells, induced in the absence of CD4 help, do not respond to secondary stimulation [17]. This dictates that the antigen should contain epitopes able to stimulate both sorts of T cells. Whole recombinant proteins or tumour cells are therefore more likely to be effective than CD8 target epitopes alone, which have been used in some human experiments.

**Escape mechanisms:** Mechanisms for escaping from immune responses are not confined to tumours. Almost, if not all, microorganisms have escape mechanisms and these are often similar to those found in tumours. Microorganisms and tumours may be immunosuppressive and these effects may be general or local. Many tumour-bearing patients show depressed immune responses and defects in signalling through the TCR and its associated CD3 complex have been demonstrated [18]. The cause of this effect is unclear but may be due to cytokines such as Transforming Growth Factor-b (TGF-b) and Vascular Endothelial Growth Factor (VEGF), often produced by tumours, which have been shown to have suppressive effects on lymphocytes. Furthermore, there is a complex relationship between tumours, their microenvironment, and the immune system, that may facilitate tumour growth and metastasis as much as preventing it [19]. A major escape mechanism of tumours, also found in microorganisms, is interference in antigen presentation. More than half of all tumours show abnormalities in MHC class-I expression, ranging from downregulation of a single allele to loss of all class-I molecules, and diverse molecular mechanisms for this have been demonstrated [20]. Clearly, however effective an antitumour immunization regime may be, it will be ineffective if the target epitopes can no longer be presented to effector T cells by the tumour cells. The common finding of loss of some HLA alleles in tumours suggests that antigen binding to as many different HLA molecules as possible should be used for immunization. Since HLA loss increases with tumour progression, active immunization is likely to be most effective if instituted as early as possible in the course of the disease. As yet this is seldom possible since conventional surgery, radiotherapy, or chemotherapy usually takes precedence over unproven modalities such as immunotherapy.

**Clinical use of mAbs**

**Naked Antibodies:** More than 200 mAbs have been tested in clinical studies, but the number of clinically relevant antibodies remains limited (Table 3). The first mAb that received US Food and Drug Administration (FDA) approval is rituximab, which is a chimeric antibody directed against the surface antigen CD20 on B lymphocytes, expressed on most B-cell NHL and subtypes of Acute Lymphatic Leukemias (ALL). In combination with polychemotherapy, rituximab is used for primary therapy of follicular NHL and diffuse large B-cell NHL as well as for maintenance therapy in recurrent follicular B-NHL after successful induction chemotherapy. Chemoimmunotherapy with rituximab is standard in therapy of primary and recurrent mantle cell lymphoma [21,22]. Rituximab might also be successful in combination with chemotherapy in CLL and in Burkitt’s lymphoma, improving progression-free and overall survival. Alemtuzumab is a humanized antibody directed against CD52 on B and T lymphocytes, and monocytes, macrophages, eosinophilic granulocytes, and NK cells. It is approved for clinical application in fludarabine-refractory CLL. In those patients, remission rates of 40% can be achieved. Interestingly, alemtuzumab has been shown to be especially effective for bone marrow manifestations of CLL. The role
of alemtuzumab in primary therapy of CLL is not yet clear. Further studies will evaluate whether the efficacy of alemtuzumab in recurrences can be enhanced. Promising results were seen with alemtuzumab-chemoimmunotherapy in periphery T-cell lymphoma [23]. In contrast to rituximab, therapy with alemtuzumab is accompanied by heavier infusion-associated complications such as fever, shivering, dyspnea, or exanthema, and a higher rate of infectious complications. Metastasized Human Epidermal Growth Factor Receptor 2 (HER2)-expressing breast cancer treatment was the first indication for trastuzumab, a HER2-specific humanized monoclonal antibody. HER2 is a receptor tyrosine kinase of the EGFR family that is overexpressed in 25-30% of all breast cancer patients. Overexpression of HER2 leads to enhanced cell proliferation. A phase III study combining trastuzumab with first-line chemotherapy showed prolonged progression-free and overall survival [24]. It has also been approved as monotherapy for chemotherapy refractory metastasized breast cancer [25]. In addition, efficacy of adjuvant chemotherapy can be [26]. The chimeric mAb cetuximab is directed against EGFR. EGFR plays an important role in pathogenesis and progression of solid tumors such as colorectal cancer, NSCLC, and head and neck tumors. Binding of cetuximab to EGFR hinders the activation of intracellular tyrosine kinases and the following signal transduction pathway. The antibody also induces direct lysis of the tumor cells. A multicenter phase II study (BOND-1) was able to show that combination of irinotecan with cetuximab could overcome irinotecan resistance. In 23% of the patients, tumor remission, and in 30% stable disease was reached [27]. Cetuximab is now used for therapy of metastasized colorectal carcinoma in combination with irinotecan after progression with irinotecan monotherapy. In a phase III study of locally advanced head and neck tumors, the combination of cetuximab with radiotherapy significantly prolonged survival [28]. In metastasized NSCLC, a phase II study showed that combination of cisplatin, vinorelbine, and cetuximab leads to a significant survival benefit compared with chemotherapy with cisplatin and vinorelbine alone [29]. Bevacizumab is a VEGF-specific humanized mAb. Binding to VEGF inhibits tumor angiogenesis. It is proved in combination with irinotecan and 5-FU for first-line therapy of metastasized colorectal carcinoma. Patients with contraindications for irinotecan can be successfully treated with 5-FU and bevacizumab.

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Target antigen</th>
<th>Structure</th>
<th>Application</th>
</tr>
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<tbody>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>Chimeric IgG-1κ</td>
<td>B-NHL Mantle cell lymphoma CLL B-precursor ALL</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>CD52</td>
<td>Humanized IgG-1κ</td>
<td>CLL Peripheral T-cell lymphomas</td>
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<tr>
<td>Trastuzumab</td>
<td>HER2</td>
<td>Humanized IgG-1κ</td>
<td>Breast cancer</td>
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<td>Cetuximab</td>
<td>EGFR</td>
<td>Chimeric IgG-1κ</td>
<td>Head and neck cancer Colorectal carcinoma NSCLC</td>
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<td>Bevacizumab</td>
<td>VEGF</td>
<td>Humanized IgG-1κ</td>
<td>Colorectal carcinoma NSCLC</td>
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Table 3: mAbs in clinical use.

In primary therapy of advanced NSCLC, the addition of bevacizumab to carboplatin and paclitaxel leads to enhanced progression-free and overall survival [30,31]. Contraindications are squamous cell histology and brain metastases because of enhanced risk of heavy bleeding.

**Radioimmunoconjugates:** With the help of immunoconjugates, cytotoxic substances such as radioisotopes, cytokines, enzymes, or toxins can specifically be targeted to the tumor cells by the monoclonal antibody. Only two radioimmunoconjugates have approval
for therapy, 90Y-ibritumomab tiuxetan and 131I-tositumomab. Both are directed against CD20 and are used for recurrent or refractory follicular B-NHL after therapy with rituximab. The radioimmunoconjugates might also be successful in therapy of transformed follicular NHL and primary diffuse large cell B-NHL.

**Conclusion and Future Directions**

The immune response to tumor cells involves a complex interplay of antigen-presenting cells, effector cells, cytokines, and chemokines that evolves over time and space. An understanding of basic immunologic principles can lead to insights into the reasons for failure of the endogenous antitumor immune response and an opportunity to manipulate components of the immune system to augment an antigen-specific effect. The identification of tumor antigens capable of eliciting immunity was one of the first steps toward achieving this goal. Now, an understanding of the mechanisms of T-cell recognition and co-stimulation has led to the possibility of vaccinating patients using DCs and the development of methods to isolate and expand antigen-specific CD4 and CD8 T cells *ex vivo* for adoptive transfer. The role of cytokines and chemokines in bringing together many of these effectors of innate and adaptive immunity yields yet another opportunity to augment the antitumor immune response. The use of monoclonal antibodies, which are already in clinical use, foreshadows the evolution of immunotherapy as a more broadly applied modality for the treatment of patients with cancer. The potential synergy of combining immunotherapy with chemotherapy, cytokines and chemokines with a tumor-specific vaccine, or antiangiogenic antibodies with adoptive T-cell therapy, may provide additional weapons in the anticancer armamentarium. The application of immunotherapy at earlier stages of malignancy may provide the opportunity for more complete and durable response in patients for whom more conventional therapy would be ineffective. However, many questions remain unanswered. For example, what is the significance of regulatory T cells in tumor immunity? What is the best strategy for optimal vaccination? What phenotypic qualities are desired of effector cells for adoptive therapy? How can cytokines and chemokines be integrated into the use of vaccines in delivering immunogens to the site of activation and augmenting the ensuing response? What triggers cells down the path of immunologic memory to ensure long-term immunoprotection? How can we identify and address obstacles of immune escape? With more precise immunologic tools and preclinical models at our disposal, it is hoped that many of these questions can be answered.

**References**


Cancer Statistics

Cancer is a leading cause of disease worldwide and it is estimated that 12.7 million new cancer cases occurred worldwide in 2010. Lung (1.6 million, 12.7% of the total for men and women), female breast (1.4 million, 10.9% of the total for women), colorectal (1.2 million, 9.7% of the total for men and women) and stomach cancers (1 million, 7.8% of the total for men and women) were the most common, accounting for more than 40% of all cases diagnosed [1].

Currently, one in 3 women and one in 2 men in the United States will develop cancer in his or her lifetime. Increases in the number of individuals diagnosed with cancer each year, due in large part to aging and growth of the population, as well as improving survival rates, have led to an ever-increasing number of cancer survivors. The goal of treatment is to “cure” the cancer, or prolong survival in patients with advanced disease, while preserving the highest possible quality of life in both the long and short term [2]. Lung cancer has been the leading cause of malignancy in women since 1987, when it surpassed breast cancer. In 2010, it was estimated that over 116,000 men and 105,000 women would be diagnosed with lung cancer in the United States. Lung cancer is responsible for over 71,000 deaths per year in women. This number exceeds the mortality associated with both breast cancer (39,840 deaths) and colon cancer (24,790 deaths) combined, which are the second and third leading causes of cancer-related mortality in women, respectively [3]. Thus demands for efficient therapy are needed to control the growth and multiplication of cancer.

Cancers of the lung and bronchus, prostate, and colorectum in men and cancers of the lung and bronchus, breast, and colorectum in women continue to be the most common causes of cancer death. These 4 cancers account for almost half of the total cancer deaths among men and women (Figure 1). In 2012, lung cancer is expected to account for 26% of all female cancer deaths and 29% of all male cancer deaths. The data represented in Figure 1 revealed the most common cancer expected in men and women in 2012. In men, lung, prostate, bronchus and colorectum account 50% of all newly diagnosed cancer. In women, lung, breast, bronchus and colorectum also account the other 50% [4].
Apoptosis

Most of cancer chemotherapeutics and chemopreventives exert their effects by triggering either apoptotic cell death or cell cycle transition, and accordingly, the induction of tumor cell apoptosis is used to predict tumor treatment response [5]. Apoptosis is a selective process of physiological cell deletion that plays an important role in the balance between cellular replication and death. Apoptotic signaling can proceed via two pathways, i.e., via death receptors expressed on the plasma membranes of cells or alternatively via mitochondria, which contain several proteins that regulate apoptosis. The death receptor pathway is initiated by the ligation of membrane bound Tumor Necrosis Factor (TNF) or Fas receptors, which result in a caspase-8-dependent cascade and subsequent cell death. During this cascade, caspase-8 cleaves Bid and induces cytochrome c release and/or directly activates caspase-3 [6-8]. On the other hand, the ‘intrinsic’ (or mitochondrial) pathway, which is triggered by diverse cellular stresses, such as cytokine deprivation, DNA damage or oncogene activation. The mitochondrial pathway involves cytochrome c release, which leads to caspase-9 activation and a proteolytic caspase cascade. After reception of death signals, the outer mitochondrial membrane becomes permeable leading to a cascade of events. Cytochrome-c is released and subsequently activates caspase-9, which then activates caspase-3 that acts on specific apoptotic substrates these pathways are largely independent, each activating different initiator caspases, but they are connected through the cleavage of the BH3 only protein BID [9]. The two molecular mechanisms extrinsic and intrinsic phases of apoptosis are outlined in (Figure 2).

**Figure 2:** Schematic representations of the main molecular pathways leading to apoptosis.
There are two main pathways to initiate apoptosis. In the extrinsic pathway upon ligand binding to specific receptors the DISC complex is formed and caspase 8 activated. In the intrinsic pathway, the release of cytochrome c from the mitochondria results in the formation of the apoptosome and activation of Caspase 9. Caspase 8 and 9 then activate downstream caspases such as caspase 3 resulting in cell death [10].

**p53 and Mdm2**

The p53 tumor suppressor plays a pivotal role in regulating cellular processes including cell cycle arrest, apoptosis, cell metabolism and senescence. Mutation of the TP53 gene or inactivation of the p53 signaling pathway occurs at a high frequency in many human tumors, suggesting that p53 plays a critical role in preventing normal cells from becoming cancerous. p53 is a stress-inducible protein; it is inactive under normal physiological conditions and activated in response to various types of stresses such as DNA damage and ribosomal stress [11]. Activated p53 can either induce cell cycle arrest or inhibit cell growth or promotes cell apoptosis depending on different type of stress and the cellular context. Multiple mechanisms have been revealed to collectively accomplish the regulation of p53 activity which ultimately determines the selectivity of p53 for specific transcriptional targets, resulting in precise control of the p53 activity. p53 is the most frequently inactivated tumor suppressor gene in human cancer. Clinical studies have shown that p53 is mutated in approximately 50% of human cancers [12,13].

Mdm2 (Murine double minute 2) was discovered on double minute chromosomes in a derivative cell line of NIH-3T3 cells [14,15]. Mdm2 belongs to the family of E3 ubiquitin ligases that contain a RING domain16 and serves as the major E3 ubiquitin ligase for p53 degradation. Several studies have illustrated the importance of Mdm2 in the control of p53 activity. The mechanism by which Mdm2 suppresses p53 has classically been thought to occur by two distinct ways: by binding to the N-terminal domain of p53 and masking p53’s access to transcriptional machinery, and by ubiquitinating p53 and targeting it for proteasomal degradation [16-20]. However, it was found that Mdm2-p53 binding alone in the absence of Mdm2 E3 ubiquitin ligase activity is insufficient to suppress p53 activity [21]. MdmX has been identified as a highly homologous gene that is closely related to Mdm2 [22,23]. Similarly to Mdm2, MdmX possesses a p53 binding domain at its N-terminus and a RING finger domain at its C-terminus through which it heterodimerizes with Mdm2. However, unlike Mdm2, MdmX does not have appreciable ubiquitin ligase activity. Because of its sequence similarity with Mdm2 and its ability to inhibit p53-induced transcription when overexpressed, MdmX has been hypothesized to act as a negative regulator of p53 through physical binding [24].

In response to stress, a decrease in Mdm2 protein levels and/or its activity and the interaction between Mdm2 and p53 lead to p53 stabilization. The mechanisms by which p53 escapes the detrimental effects of Mdm2 binding vary depending on the type of stress signals. The increase in p53 levels and in transcriptional activity of p53 leads in turn to increased production of Mdm2 (Figure 3). Elegant quantitative studies of p53 show that while an individual cell may have only one pulse of p53 activity, its neighbor might have several repeated pulses [25]. As the amount of radiation increases, the percentage of cells showing a high number of p53 pulses also increases. Interestingly, the mean height and width of a pulse is constant and independent of the damage level. Sister cells continue to oscillate in a correlated way even after cell division. These intriguing observations open new questions regarding the mechanism and function of p53 oscillatory dynamics, including the reason for the observed variation between cells [26]. Understanding why some cells respond poorly is of critical importance as these genetically unstable cells will continue to proliferate
and eventually become the target of additional oncogenic mutations and the cell-of-origin of tumor development.

Figure 3: Regulation of p53 by Mdm2.

Prior to DNA damage, Mdm2 interacts with both p53 leading to their ubiquitination and targeting for proteasomal degradation. Mdm2 bound to p53 has been localized to regulatory regions of a number of p53 target genes, leading to repression of their expression. Upon DNA damage, both Mdm2 and p53 become post-translationally modified such that they no longer interact. p53 is now capable of influencing gene expression [27].

p53 as Target for Cancer Therapy

Cancer is usually associated with aberrant cell cycle progression and defective apoptosis induction due to the activation of proto-oncogenes and/or inactivation of tumor suppressor genes [28]. Given the central role of p53 in cancer prevention and suppression and in chemosensitization or radiosensitization, p53 has to be abrogated during carcinogenesis for most cancers to arise. Indeed, p53 is inactivated by point mutations in more than 50% of human cancers with a majority of mutations occurring in the DNA binding domain, which either change wt p53 conformation (conformation mutants, e.g., 175H, 249S, 281G) or abolish its DNA contact (contact mutants, e.g., 248W, 273H) [29]. Most malignant tumors that disrupt p53 signaling pathways remain addicted to p53 mutants. Various strategies have been successfully developed to reconstitute p53 functions in order to abrogate tumor progression [30,31]. Based on the action sites, these strategies can be briefly listed into three groups: replacement of wild-type p53 by gene therapy, augmenting of wild-type p53 by inhibition of MDM2-mediated degradation and reactivation of mutant p53 by alteration of protein conformation [32], the three major strategies to target the tumor suppressor p53 are outlined in (Figure 4).

Ceramide modulates the expression of p53 to resuscitate wild-type p53 (phosphorylated, red fluorescence in the cell nucleus) and p53-dependent apoptosis, thus sensitizes mutant p53 tumors to therapies. Silencing of glucosylceramide synthase (GCS, Green Fluorescence in Golgi apparatus) with MBO-as GCS disrupts ceramide glycosylation to enhance endogenous ceramide. (+) - Increasing enzyme activity or synthesis; (−) - Inhibiting enzyme activity or synthesis; * - These genes are upregulated by mutant p53 in cancer cells.
A promising example of small molecules that restore the function of mutant p53 is PRIMA-1, p53 Reactivation and Induction of Massive Apoptosis (PRIMA)-1 is a small molecule that binds covalently with thiol groups in mutant p53 and restores DNA-binding activity to some mutant p53 proteins. PRIMA-1 preferentially suppresses the growth of tumor cell lines containing mutant p53, indicating that it functions by acting on mutant p53. PRIMA-1 is in a Phase I clinical trial [33]. The PRIMA-1 (p53 reactivation and induction of massive apoptosis) restores wild-type conformation to mutant p53 protein by covalent binding to and modifying the thiol groups of His175 and His273 in the core domain. The more potential PRIMA-1 analogue APR-246 that inhibits human tumor growth and is able to synergize with chemotherapeutic drugs is currently tested in a clinical trial [34]. The restoration of the impaired function of the p53 protein by disrupting the Mdm2–p53 or Mdmx–p53 interaction offers a fundamentally new avenue for the treatment of a broad spectrum of cancers [35,36]. The search for efficient p53–Mdm2 inhibitors has led to several small-molecule ligands. The most thoroughly studied among them is Nutlin-3 [37].

In conclusion, in cancer carrying a wt. p53, p53 is often nonfunctional as a result of either being degraded by overexpressed Mdm2 or being excluded from the nucleus where p53 acts as a transcriptional factor. There are various approaches targeting p53 1) to activate wt. p53, 2) to reactivate mutant p53 or selectively kill cancer cells with mutant p53, and 3) to temporarily inhibit wt. p53 for normal cell protection. Successful clinical development of these three classes of novel compounds would eventually revolutionize the current cancer treatment.
therapies to benefit a majority of cancer patients. The approaches to activate p53 include the use of nongenotoxic small molecules to activate endogenous wt-p53, chemoradiation to activate endogenous wt. p53, of gene therapy to introduce wt. p53 or modified adenovirus to kill cancer cells with mutant p53, and of synthetic peptides [38] (Figure 5).

As illustrated in the figure, three classes of p53 targeting compounds have been identified and characterized. The first classes are the compounds that activate or restore wild-type p53 function and can be used in human cancers harboring a wt. p53. The second class of compounds reactivates and rescues the mutant p53 with an application in human cancers carrying a p53 mutation. The third class is capable of inhibiting wt. p53 and can be used during chemoradiation to block p53 activation in normal cells, thus reducing cytotoxicity [38].

**Mdm2 Targeted Drugs**

These results have provided an encouraging direction for p53-target therapeutic strategy utilizing inhibition of MDM2. There are three main categories of MDM2 inhibitors: inhibitors of MDM2-p53 interaction by targeting to MDM2, inhibitor of MDM2-p53 interaction by targeting to p53, and inhibitors of MDM2 E3 ubiquitin ligase [39]. The binding sites and mechanism of action for these inhibitors are further illustrated in Figure 6.
Nutlins, consisting of nutlin 1, 2 and 3, analogs of cis-imidazoline, fit in the binding pocket of p53 in MDM2 and inhibit the interaction between MDM2 and p53. Nutlin-3, an analog of the series, has the most potent binding capacity and lowest inhibition concentration, induced p53 levels, and activated p53 transcriptional activity. Nutlin-3 has been shown to exhibit a broad activity against various cancer models with wild-type p53 including cell lymphoma [40]. The major modes of inhibition of p53-mdm2 interaction by small molecules are outlined in Figure 7.

a) The p53 protein binds to Mdm2/x using a short helix with three hydrophobic residues Phe19 (orange), Trp23 (blue), and Leu26 (green) which fills the binding cleft.

b) Nutlin-2 is a close analogue of the most-studied Mdm2 inhibitor Nutlin-3.

c) Imidazole-indole compound WK23 in complex with Mdm2. WK23 possesses a 6-chloroindole group which is bound to Mdm2 in the same way as the Trp23 side chain of p53.

d) Benzodiazepinedione inhibitors utilize para-halogenated phenyl rings similar to those of the Nutlins. The Phe19 pocket is filled by the 7-iodobenzene ring.

e) A diastereomer of MI-63 positions the 6-chlorooxindole group in the Trp23 pocket. The Phe19 pocket interacts with the neopentyl group of the inhibitor and the 2-fluoro-3-chlorophenyl is situated in the Leu26 pocket.

f) Chromenotria-zolopyrimidines are also equipped with two halogenated phenyl rings that fill Trp23 and Leu26 pockets in a “Nutlin-like” fashion.

g) The imidazole-indole compound WW298 in complex with Mdmx. The 6-chloroindole group binds to Mdmx in the same way as the Trp23 side chain of p53 does. Note that in (c), (e), and (g) the 6-chloroindole group is used to bind in the Trp pocket, and that in (b), (d), and (f) a 4-halogenphenyl serves the same purpose [41].

Figure 7: Low-molecular-weight inhibitors of p53–Mdm2/x binding (b-g, different categories for inhibition of interactions)
Ceramide as Novel Messenger of Death

Ceramides are a family of lipids that consist of sphingosine covalently linked to a fatty acid. Though ceramides were previously believed to be merely structural components of the cell membrane, discoveries over the last few decades reveal that virtually all stress stimuli (e.g., inflammatory mediators and oxidative stress) [42]. Three major pathways — de novo synthesis, sphingomyelin hydrolysis, and the salvage pathway — account for the production of ceramide within the cell [43] (Figure 8).

Recent studies revealed that another important ceramide binding protein, CERT, which specifically transports ceramide from the ER to the trans-Golgi for SM synthesis, plays a role in cancer drug resistance [44]. One of the well-described downstream targets of ceramide has been the protein phosphatases of the PP2A and PP1 family, also known as Ceramide-Activated Protein Phosphatases (CAPPs) [45]. PP2A is a tumor suppressor in cancer, and its activation regulates various downstream oncoproteins [46]. However, how ceramide mediates the activity of PP2A has been elusive. Most recently, ceramide was shown to directly bind to the oncoprotein SET/PP2A inhibitor 2 (I2PP2A) in A549 lung cancer cells. SET/I2PP2A is a nuclear protein and a known inhibitor of PP2A activity. Thus, this discovery gave insight into the possible mechanism by which ceramide regulates PP2A activity via binding to its biological inhibitor (SET/I2PP2A), which controls PP2A activity and its downstream targets, such as proto-oncogene c-Myc [47] thus describing a novel mechanism for regulating PP2A-dependent antiproliferative roles of ceramide (Figure 9).

In normal cells, ceramide and its binding protein, I2PP2A, which is the inhibitor for PP2A, are mostly in a 1:1 ratio. Therefore, it is believed to be the binding and inactivation of ceramide by I2PP2A that liberates the active form of PP2A, which, in turn, acts upon c-Myc, leading to the dephosphorylation and degradation. In cancer cells, elevated levels of I2PP2A were observed, which inhibits most of the available PP2A and results in stable (active) oncogenic c-Myc. The stable form of c-Myc can mediate tumor growth and cancer
progression by upregulating expression of several oncogenes. I2PP2A: Protein phosphatase 2A inhibitor 2; PP2A: Protein phosphatase 2A [48].

Figure 9: Regulation of oncogenic c-Myc by protein phosphatase 2A via control of ceramide in normal and cancer cells.

Ceramide promotes apoptosis through the mitochondrial pathway, in part due to its effects on Bcl-2 family proteins [49]. Treatment of A549 lung adenocarcinoma cells with cell-permeable ceramide and/or agents that induce the synthesis of de novo ceramide downregulated Bcl-x (L) mRNA and protein levels and concomitantly increased Bcl-x(s) mRNA and protein [50]. This effect correlated with increased sensitivity of A549 cells to daunorubicin. Furthermore, A549 cells resistant to chemotherapeutic agents and cell-permeable ceramides demonstrated increased Bcl-x (L) levels. Others have reported that UV light-induced Bax activation and ensuing cytochrome C release and apoptosis, require the actions of A-SMase. Thus, in HeLa cells treated with siRNA against A-SMase or in A-SMase−/− cells from NPD patients, UV light induction of Bax conformation change was drastically reduced. Further, restoration of A-SMase or addition of exogenous ceramide to A-SMase-deficient cells restored the UV pro-apoptotic response. These findings suggest that ceramide activates the intrinsic apoptotic pathway through its effects on Bcl-2 family proteins [51].

Apoptosis can also be activated through the extrinsic, or death receptor pathway (Figure 10). TNF receptor 1 and other members of the TNF family initiate this process when activated by ligand. Once activated, these receptors interact with an adaptor protein called FADD, leading to assembly of a protein complex that activates caspase-8, which in turn cleaves and activates the Bcl-2 family member, Bid. Bid then translocates to the mitochondrial outer membrane, initiating the intrinsic apoptotic pathway. Interestingly, Bid can also be cleaved by the lysosomal aspartate protease, capthepsin D. It was recently established that activation of cathepsin D by TNFα requires A-SMase activity. Further, ceramide was shown to bind directly to cathepsin D, causing autocatalytic proteolysis of the pre-pro-cathepsin D to form the enzymatically active isoforms of the enzyme, thereby implicating ceramide in regulation of Bid processing [52].
Figure 10: Sphingolipid effects on intrinsic and extrinsic apoptotic pathways.

The two pathways to apoptosis are shown. The extrinsic pathway begins by ligand binding to cell surface receptors such as FasR or TNFR, followed by Recruitment of Death Domain containing protein adaptors (e.g., TRADD) resulting in the formation of Death-Inducing Signaling Complex (DISC). This complex then activates the caspase cascade, culminating in cell death. The intrinsic or mitochondrial pathway begins when pro-apoptotic members of the Bcl-2 family cause mitochondrial release of cytochrome c, which binds to and activates Apaf-1 (apoptotic protease-activating factor), resulting in subsequent activation of the caspase cascade. Smac/DIABLO (Second mitochondria-derived activator of caspase/Direct Inhibitor Of Apoptosis-Binding Protein with Low pI) is also released, inhibiting XIAP. XIAP (X-linked Inhibitor of Apoptosis Protein) functions as an inhibitor of caspases 3, 7 and 9. Shown are also the influence of ceramide, A-SMase and S1P on different components of the pathway.

Antibody Drug Complexes as Target Therapy

Although the last 50 years has seen remarkable progress in the prevention, detection and treatment of cancer, the most common methods (i.e., radiation, surgery and chemotherapy) often result in serious side effects. Additional deficits of current cancer therapies include non-specific systemic distribution, non-specific suppression of rapidly dividing cell types, inadequate drug concentrations at target tissues (i.e., tumors or cancerous cells), multi-drug resistance and a limited ability to monitor therapeutic responses [53].

An ideal anti-cancer therapeutic would be one that can be selectively concentrated in cancer cells while exerting minimal effects on normal tissues [54]. To achieve this, scientists are exploring biological molecules such as mAbs designed to target receptors on cancer cells or ligands relevant to cancer pathways that will facilitate delivery of cytotoxins, radioactive isotopes or chemotherapeutic drugs. The mAbs approved by the US Food and Drug Administration for cancer treatment is listed in (Table 1). The approach of conjugating bio-active anti-cancer molecules to mAbs has some limitations, e.g., low drug to mAb conjugation ratios. Increasingly, researchers are examining NMs to overcome some of the shortcomings of immunoconjugates [54].

<table>
<thead>
<tr>
<th>Year</th>
<th>International non-proprietary name/Trade name</th>
<th>Target</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>Rituximab/Rituxan</td>
<td>CD20</td>
<td>B-cell lymphoma</td>
</tr>
<tr>
<td>1998</td>
<td>Trastuzumab/Herceptin</td>
<td>HER2</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>2001</td>
<td>Alemtuzumab/Campath</td>
<td>CD52</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>2002</td>
<td>Ibritumomab tiuxetan/Zevalin</td>
<td>CD20</td>
<td>B-cell lymphoma</td>
</tr>
</tbody>
</table>
Table 1: Monoclonal antibodies approved by the US food and drug administration for the treatment of cancer.

<table>
<thead>
<tr>
<th>Year</th>
<th>Antibody/Trade Name</th>
<th>Target/Receptor</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>Tositumomab/Bexxar</td>
<td>CD20</td>
<td>B-cell lymphoma</td>
</tr>
<tr>
<td>2004</td>
<td>Bevacizumab/Avastin</td>
<td>VEGF</td>
<td>Colon, lung, breast and renal cancer</td>
</tr>
<tr>
<td>2004</td>
<td>Cetuximab/Erbitux</td>
<td>EGFR</td>
<td>Colon, lung cancer</td>
</tr>
<tr>
<td>2006</td>
<td>Panitumumab/Vectibix</td>
<td>EGFR</td>
<td>Colon cancer</td>
</tr>
<tr>
<td>2009</td>
<td>Ofatumumab/Arzerra</td>
<td>CD20</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>2011</td>
<td>Ipilimumab/Yervoy</td>
<td>CTLA-4</td>
<td>Melanoma</td>
</tr>
</tbody>
</table>

Gemtuzumab Ozogamicin

Gemtuzumab ozogamicin (GO, Mylotarg) (Figure 11) consists of a semisynthetic derivative of calicheamicin (N-acetyl-γ calicheamicin 1,2-dimethyl hydrazine dichloride), a potent enediyne DNA-binding cytotoxic antibiotic, linked to an engineered humanized monoclonal IgG4 antibody (hP67.6) directed against the CD33 antigen present on leukemic myeloblasts in most patients with AML (80%). IgG4 has interesting properties for a carrier. It has the longest circulating half-life of all isotypes, with limited ability for complement fixation and antibody-dependent cellular toxicity. The unconjugated antibody hP67.6 is not known to be cytotoxic.

![Image of Gemtuzumab Ozogamicin](image)

Figure 11: A - Immunoconjugate binding and internalization; B - Immunoconjugate intracellular trafficking from endosome to lysosome, with linker cleavage. Acid-labile AcBut hydrazone linker is cleaved in the acid environment of lysosome. C, calicheamicin derivative is released intracellularly. The reduction to the active enediyne form requires glutathione. D, the active enediyne form binds to the minor groove in DNA and causes double-strand breaks, resulting in cell death. E, Pgp-mediated efflux may be a mechanism of drug resistance in leukemic cells.

The cytotoxic drug is attached to the antibody through a covalent linkage (condensation) of a bifunctional linker, 4-(4-acetylphenoxy) butanoic acid (AcBut linker), which allows
stability in physiologic buffers (pH 7.4) and efficient calicheamicin release inside lysosomes (pH 4). The average loading of calicheamicin on the antibody is 2.5 mol/mol (drug-loading range of 2–3 mol of calicheamicin per mole of antibody). Calicheamicin binds to the minor groove in the DNA and causes double-strand DNA breaks, resulting in cell death. The custom-made, well-controlled, hydrolysable bond with the AcBut linker showed significantly more potent and selective calicheamicin conjugates of P67.6 against HL-60 cells in vitro. This effective intracellular hydrolytic release of the calicheamicin derivative was important for its intracellular trafficking and the subsequent access to its DNA target [55]. This indicates the importance of designing linkers that are specific for the individual target cell type [56].

Targeting HER2

Human Epidermal Growth Factor Receptor 2 (HER2), also known as ErbB2, c-erbB2 or HER2/neu, is a 185 kDa protein (p185) with an intracellular tyrosine kinase domain and an extracellular ligand binding domain. In humans, HER family includes four structurally related members, HER1 (ErbB1, also known as EGFR), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4). Although HER2 is the only receptor which has no identified ligand, it is the preferred partner to form heterodimer with other HER members. HER2 involved heterodimerization is the most potent signal transduction pathway among all dimmers formed by the HER family [57]. HER2 plays important roles in cell growth, survival, and differentiation in a complex manner. The major signaling pathways mediated by HER2 involve mitogen-activated protein kinase (MAPK) pathway and phosphatidylinositol 3-kinase (PI3K) pathway. As a key gene for cell survival, HER2 gene amplification and protein overexpression lead to malignant transformation [58]. It directly associates with poor clinical outcomes in breast, ovarian, gastric, and prostate and other cancers.

Signaling occurs through both homo- and heterodimeric HER complexes (Figure 12) and can induce cell proliferation, motility, and invasion. Dysregulated expression and activity of HER-family members is prevalent in human neoplasia [59]. Strikingly, up to 30% of breast carcinomas overexpress HER2, frequently as a consequence of genomic amplification of a region of the long arm of chromosome 17 (17q21) that includes the HER2 locus. HER2 overexpression may be more frequent in ER-negative than ER-positive cancers, drives aggressive disease, and thus represents an important therapeutic target. The humanized monoclonal antibody trastuzumab (Herceptin) was the first agent developed for HER2 targeting and has dramatically improved outcomes among women with HER2-positive (defined by HER2 overexpression and/or amplification) breast cancer [60].

Conclusion

One of the major areas in cancer research is targeted delivery of drugs to cancerous cells, which can not only increase the therapeutic efficacy but also reduce the adverse side effects of the drugs [61,62]. Drugs or treatment strategies that can restore the apoptotic signaling pathways towards normality have the potential to eliminate cancer cells, which depend on these defects to stay alive. Many recent and important discoveries have opened new doors into potential new classes of anticancer drugs. The first report of p53 gene therapy in 1996 investigated the use of a wild-type p53 gene containing retroviral vector injected into tumor cells of non-small cell lung carcinoma derived from patients and showed that the use of p53-based gene therapy may be feasible [63]. As the use of the p53 gene alone was not enough to eliminate all tumor cells, later studies have investigated the use of p53 gene therapy concurrently with other anticancer strategies. For example, the introduction of wild-type p53 gene has been shown to sensitize tumor cells of head and neck, colorectal and prostate cancers and glioma to ionizing radiation [64].
Cancer prevention is entering a new chapter in targeted therapies and personalized medicine due to the advance of molecular biology and medicinal chemistry. Most likely, several compounds from this review will be approved for clinical application. Although the reports to date suggest that substantial potential exists for mAb combinations as future cancer therapies, there are numerous logistical hurdles that must be overcome. The mAb therapies currently marketed are costly in part because of the large investment necessary for their development. Despite the challenges, innovative ways of combining biologic therapies are emerging. The ability to produce recombinant “polyclonal-like” antibodies is one mechanism for producing complex mixtures of antibodies to treat complex diseases. Research designed to deliver combinatorial targeted therapies appears to be moving rapidly toward multi-specific applications.
antibody-like therapeutics, for example, bispecific antibodies have been reported for some time [65,66]. The future design of potential drug combination therapies and the follow-up of their outcome will undoubtedly be facilitated by gene profilings. As the clinical trials of these inhibitors progress, more efforts should be directed to further unravel the complex biology and genetics, and the crosstalk signals of the cancer cells. Many questions remain to be answered:

(1) What are the long-term safety and toxicities of these inhibitors?

(2) How to use biomarkers to select patients who will benefit most from these inhibitors?

(3) How to combine these targeted therapies with cytotoxic agents or other treatment modality such as radiation?

References


Endometrial cancer is the most common gynaecological malignancy in western countries. Continued investigation into the molecular pathways of endometrial cancer development and progression have enhanced the existing knowledge of this disease process and have led to the discovery of novel, superior treatment options for patients. This chapter reviews the molecular targets of endometrial cancer with particular emphasis on targeting the members of tyrosine kinase receptors, mTOR pathway and numerous agents such as microtubule stabilizing agents, Histone Deacetylase Inhibitors that are in clinical evaluation for inhibiting the tumorigenesis. The mechanism of action, pre-clinical and clinical trial data of these agents and the current status thereof have been discussed in detail. Although the indication of targeted therapies in endometrial cancer is still under evaluation, it is possible that in near future, it is required to assess molecular features of endometrial cancer (particularly for widely disseminated and recurrent tumors) to suggest the most appropriate approach to treat the patient.

Keywords: Endometrial Cancer; EGFR; Histone Deacetylase Inhibitors; mTOR; Tyrosine Kinase Receptor

Abbreviations

- 4EBP-1  4E-Binding Protein-1
- AMPPK  AMP- Activated Protein Kinases
- CDK1  Cyclin-Dependent Kinase 1
- EGF  Epidermal Growth Factor
- EGFR  Epidermal Growth Factor Receptor
- ErbB-2  Epidermal Growth Factor Type II Receptor
Introduction

Endometrial carcinoma is the most common gynecologic malignancy in the US. In 2012, it was estimated that there would be over 47,130 new cases and more than 8010 endometrial cancer related deaths making endometrial cancer an important health issue for women [1,2]. Endometrial cancer refers to several types of malignancy which arise from the endometrium, or lining of the uterus. This corresponds to a lifetime risk of 2.6% for women living in developed nations; with a median age at diagnosis of 61 years [3,4]. The epidemiology of endometrial cancer is multifactorial. About 90% cases of endometrial cancer are sporadic, whereas the remaining 10% cases are hereditary [5]. The most common risk factors associated with the development of endometrial carcinoma are prolonged exposure to endogenous or exogenous estrogens and obesity [6]. Unopposed estrogen replacement therapy and the use of tamoxifen are the most common sources of exogenous estrogen, whereas endogenous sources such as obesity, cirrhosis, estrogen-producing tumors, and reproductive factors such as anovulation are also associated with the development of endometrial carcinoma [7]. A smaller subset of sporadic cancers is associated with aging and unique genetic/molecular changes, producing a more aggressive variant, serous/clear
cell type. Bockman in 1983 first described the two main clinicopathological variants of endometrial carcinoma—type I and type II.

Type I endometrial cancers represent the majority of sporadic cases of endometrial cancer, accounting for 70% to 80% of new cases [8,9]. These cancers are primarily associated with unopposed estrogen exposure and diagnosed in pre- and post-menopausal women. Type I endometrioid lesions arise in a background of hyperplasia and commonly express estrogen and progesterone receptors [4,8]. Clinically, type 1 cancers are more often low-grade endometrioid tumors, well differentiated and therefore carry a good prognosis. It is associated with conditions that elevate estrogen levels. Some of the conditions may result in hyperestrinism, diabetes, liver disease, obesity, infertility and menstrual cycle disorders [10]. In contrast, type II endometrial cancers are less common, accounting for 10% to 20% of endometrial cancers [9,11]. Type II endometrial cancers are estrogen independent i.e. not associated with increased exposure to estrogen and diagnosed mostly in older and post-menopausal women. They are often of non-endometrioid cancer, high-grade histology, usually papillary serous or clear cell carcinoma. Clinically, type II cancers are marked by an aggressive clinical course, and they have a propensity for early spread and poor prognosis.

Aside from their morphologic and clinical features, type I and type II endometrial cancers are further notable by genetic alterations (Figure 1).

Endometrioid and non-endometrioid cancers are associated with mutations from independent sets of genes [12]. Microsatellite instability associated with defects in DNA mismatch repair genes, phosphatase and Tensin Homolog Deleted on Chromosome 10 (PTEN) gene silencing, and mutations in the K-ras and β-catenin genes are the major alterations in endometrioid carcinomas which are estrogen-dependent. PTEN and K-ras mutations and microsatellite instability are early molecular events, developing in a subset of atypical endometrial hyperplasias, whereas p53 gene mutation is a late event occurring during the progression of 10-20% of endometrioid carcinomas [6,8,9,11,12]. Nonendometrioid endometrial cancers frequently show p53 mutation, mainly represented by Uterine Papillary Serous Carcinoma (UPSC), and followed by the inactivation of p16 and e-cadherin and the amplification of Epidermal Growth Factor Type II Receptor (ErbB-2) aneuploidy and p53 mutations [6,8,9,11,12]. Despite advances that have been made in the early detection and treatment of this disease, endometrial cancer still remains a women problem both the annual incidence and the death rate associated with this disease appear to be rising [13]. This article reviews the molecular basis of treatment strategies and implication for targeted therapies for endometrial cancer.

**Treatment Options for Advanced and Recurrent Disease**

The endocrine therapy (hormonal therapy) was introduced for early stage endometrial cancer as large number of tumors was found to be estrogen dependent. Subsequently, chemotherapy approach was utilized. Initially, various cytotoxic agents were employed for
the treatment of endometrial cancer. Four active agents have been identified in Phase II trials include doxorubicin, cisplatin, carboplatin and paclitaxel (Table 1) [14-22].

<table>
<thead>
<tr>
<th>Agent</th>
<th>Structure</th>
<th>Dose</th>
<th>Response rate</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td><img src="image" alt="Doxorubicin structure" /></td>
<td>60 mg/m²</td>
<td>37%</td>
<td>Thigpen et al., 1979 [14]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 mg/m²</td>
<td>22%</td>
<td>Thigpen et al., 1985 [20]</td>
</tr>
<tr>
<td>Cisplatin</td>
<td><img src="image" alt="Cisplatin structure" /></td>
<td>50 mg/m²</td>
<td>20%</td>
<td>Thigpen et al., 1984 [18]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50, 70, 100 mg/m²</td>
<td>42%</td>
<td>Seski et al., 1982 [17]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 mg/m²</td>
<td>36%</td>
<td>Tropé et al., 1980 [16]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 mg/kg</td>
<td>31%</td>
<td>Deppe et al., 1980 [15]</td>
</tr>
<tr>
<td>Carboplatin</td>
<td><img src="image" alt="Carboplatin structure" /></td>
<td>400 mg/m²</td>
<td>30%</td>
<td>Green et al., 1990 [21]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300–400 mg/m²</td>
<td>28%</td>
<td>Long et al., 1988 [19]</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td><img src="image" alt="Paclitaxel structure" /></td>
<td>200–250 mg/m²</td>
<td>37%</td>
<td>Ball et al., 1996 [22]</td>
</tr>
</tbody>
</table>

Table 1: Single-agent activity in endometrial cancer.

These agents were non-selective and produced response rates of greater than 20% by causing DNA damage, interference with DNA repair mechanisms and disturbance of metabolic pathways [23]. Soon after combination strategy was formulated as the simultaneous combination of two or more agents provided better results [24]. To date the results of treatment of advanced and/or metastatic endometrial cancer with either chemotherapy and/or hormone therapies have been quite disappointing in adjuvant setting [25-33]. The unsatisfactory results obtained with conventional pharmacological treatment encourage further biological and clinical investigations addressed to a better understanding of specific cell targets and signaling transduction pathways involved in endometrial carcinogenesis. With the increasing understanding of cellular processes at molecular level, focus was later directed towards the development of targeted therapies for more efficacious treatment of endometrial cancer. In the subsequent sections of this review, we have discussed various molecular targeted therapies under clinical trials as mono- or combination therapies.

**Molecularly targeted therapy**

Recent advances in the understanding of molecular and genetic events leading to the development in endometrial cancer have led to the development of targeted anticancer therapies. Drug targets may focus on genes that affect apoptosis, signal transduction, epigenetic modification, drug resistance, protein folding and degradation, cell cycle progression, hormone receptor activity, and angiogenesis [34-36]. The drugs involved in molecular targeted therapy include small molecular weight inhibitors, monoclonal antibodies,
and antisense and gene therapy [37]. These new targeted agents are being investigated either alone or with conventional therapy in the treatment of endometrial cancer, as detailed below.

**Mammalian target of rapamycin (mTOR) inhibition**

There are numerous signalling pathways that are good candidates for targeted therapy in endometrial cancer [38,39]. Earlier, it was mentioned that Dysregulation of PI3K (phosphatidylinositol-3-kinase)/AKT (serine/threonine-specific protein kinase) pathway is the most frequent abnormal signalling pathway in type I endometrial cancer. Loss-of-function or mutations in PTEN result in hyperactivity of the PI3K pathway [40]. The PI3K-AKT-mTOR signaling cascade plays a critical role in cell cycle progression, cell survival, apoptosis and angiogenesis, and therefore an alteration of this pathway can contribute to neoplastic transformation (Figure 2) [41,42].

![Figure 2: Simplified schematic illustration of the PI3Kinase pathway highlighting potential downstream cellular and tissue effects of PI3Kinase signaling inhibition. The action sites for PI3Kinase and mTOR inhibitors are depicted in square boxes. The Abbreviations Used Are Phosphatidylinositol-3 Kinase (PI3K), phosphatase and tensin homolog deleted on chromosome 10 (PTEN), Mammalian Target of Rapamycin (mTOR), eukaryotic initiation factor 4E-Binding Protein-1 (4EBP-1) and Ribosomal Protein S6 Kinase (S6K).](image)

The key role of PI3K/AKT-mTOR survival pathway in endometrial cancer raises the possibility that PI3K inhibitors, such as wortmannin and derivatives, may be used as potential anticancer agents. However, the mTOR pathway can also be activated by other mechanisms including activation of Tyrosine Kinase Receptors (Epidermal Growth Factor Receptors EGFR1-4, PDGFR, KIT, IGFR ) and Ras. Moreover, the loss of function of p53 may also result in activation of mTOR. Therefore, mTOR inhibition appears to be a logical molecularly targeted therapy for this malignancy [43]. TOR proteins have pleiotropic functions, and participate in regulation of cell growth and proliferation, based on amino acid and nutrient availability or growth factor stimulation via regulation of translation and transcription. AKT activates mTOR via direct phosphorylation of tuberous sclerosis complex TCS2 (tuberin) and by the inhibition of AMP- Activated Protein Kinases (AMPPK), thereby activating Rheb and mTOR-Raptor activity. The key mTOR effectors are eukaryotic initiation factor 4E-Binding Protein-1 (4EBP-1) and ribosomal protein S6 Kinase (S6K) [44]. Through these biochemical mechanisms, mTOR enhances the translation of mRNAs encoding key proteins for cell growth and angiogenesis, such as cyclin D1, c-myc, matrix metalloprotease and Vascular Endothelial Growth Factor (VEGF) [45,46]. mTOR inhibitors (rapamycin and
rapamycin derivatives) have been recently developed as potential anticancer agents. A Phase II trial evaluating an mTOR inhibitor for the treatment of recurrent endometrial cancer has been initiated [43]. Some analogues of rapamycin, tensirolimus (CCI-799), everolimus (RAD-001) and ridaforolimus (AP-23573, ARIAD), have been tested in endometrial cancer (Table 2) [36,47].

<table>
<thead>
<tr>
<th>Intervention/Agent</th>
<th>Structure</th>
<th>Class of mTOR Inhibitor</th>
<th>Type of therapy</th>
<th>Health authority</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 CCI-779 (Temsirolimus)</td>
<td></td>
<td>Rapamycin ester; converted to rapamycin in vivo</td>
<td>Metastatic or Locally Advanced Recurrent Endometrial cancer</td>
<td></td>
<td>[48]</td>
</tr>
<tr>
<td>4 AP23573 (ridaforolimus)</td>
<td></td>
<td>Small molecule</td>
<td>Recurrent or Persistent Endometrial cancer</td>
<td>United States: Food and Drug Administration</td>
<td>Colombo N, 2007 [50]</td>
</tr>
</tbody>
</table>

Table 2: mTOR Inhibitor for endometrial cancer treatment undergoing phase II clinical trial.

Combinations of mTOR inhibitors with hormonal therapy, chemotherapy, or other targeted therapies such as Epidermal Growth Factor Receptor (EGFR) inhibitors and antiangiogenic agents have shown such promise, in the preclinical setting, several trials are currently underway to develop (Table 3) [35,51,52]. It has been shown that exposure of endometrial cancer cell lines to an mTOR inhibitor increases progesterone mRNA expression and inhibits ER mRNA expression [35,53].

Temsirolimus (CCI-799; Wyeth Pharmaceuticals) is a water soluble analogue of rapamycin...
that incorporates a bis-hydroxymethyl-propanoic acid ester. CCI-779 was designed to increase the solubility of rapamycin making this compound readily available for intravenous formulation. It can be converted to rapamycin in vivo. Like rapamycin, CCI-779 binds to intracellular immunophilin FKBP12 and is an mTOR inhibitor with potent antitumor activity in vitro and in xenograft models [54]. CCI-779 inhibits the growth of a wide range of human cancers in animal models [39,55,56]. Oza and coworkers conducted a phase II study to evaluate the activity of CCI-779 given intravenously at the dose of 25mg/week in patients with advanced or recurrent endometrial cancer. Of 19 patients evaluable for response, five (26%) had a partial response and 12 (63%) had stable disease, irrespective of PTEN status [57,48]. The most common side effects were pneumonitis, mucositis, gastrointestinal symptoms, fatigue and pain. CCI-799 is currently being tested with topotecan, bevacizumab and progestin therapy (Table 2 and 3) [35,58].

**Everolimus (RAD-001)**

Everolimus (RAD001; Novartis Pharmaceuticals), 40-O-(2-hydroxyethyl)-rapamycin, an orally bioavailable derivative of rapamycin, exerts antiproliferative effects against human tumor-derived cells growth either in culture or in animal models [59-61]. RAD001 has high affinity for an intracellular receptor protein, FKBP-12. The resulting FKBP-12/RAD001 complex then binds with mTOR to inhibit downstream signaling events. The immunosuppressive activity of RAD001 in vitro is about 3-fold lower than rapamycin, but its activity in vivo seems comparable to rapamycin due to some more favourable pharmacokinetic properties. A phase II clinical study conducted by Slowmovitz et al., showed clinical benefit response rate of 21% at 20 weeks with single agent RAD001 in pretreated patients with recurrent endometrial cancer. Loss of PTEN may predict clinical benefit response (Table 2) [61,62]. Studies carried out using knockout mice model revealed that RAD001 was able to decrease the progression of endometrial hyperplasia in the PTEN± murine model through decreased cell proliferation and increased apoptosis. High-grade hyperplasia occurred in a significantly greater percentage of the untreated PTEN± mice (80%) compared with RAD001-treated PTEN± mice (20%) [49].

A Phase I trial of everolimus used in combination with topotecan for the treatment of advanced endometrial cancer is currently recruiting (ClinicalTrials.gov) (Table 3) [62].

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Intervention/Agent</th>
<th>Type of therapy</th>
<th>Phase of clinical trial</th>
<th>References</th>
</tr>
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<tr>
<td></td>
<td>b. Bevacizumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. Topotecan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>a. Temsirolimus</td>
<td>Advanced endometrial Cancer</td>
<td>Phase I</td>
<td>Oza AM, 2009</td>
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<tr>
<td></td>
<td>b. Paclitaxel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c. Carboplatin</td>
<td></td>
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</tbody>
</table>

Table 3: Combination of mTOR inhibitors with other agents for endometrial cancer.

**Ridaforolimus (AP-23573, ARIAD)**

Ridaforolimus (AP23573; Ariad Pharmaceuticals, formerly known as Deforolimus) is a novel non-prodrug rapamycin analogue with a non-linear pharmacokinetic behavior. AP23573 was found to be stable in organic solvents, aqueous solutions at a variety of pH, and in plasma and whole blood, both in vitro and in vivo. These stability studies along with in vitro metabolism studies also indicated that AP23573 is not a rapamycin pro-drug [63]. AP-23573 has demonstrated antiproliferative activity against several human tumor cell lines in vitro including endometrial cancer cell lines, and against xenograft animal tumor models in vivo [57,64]. A phase II study assessed the activity of AP-23573 at the dose of 12.5 mg in advanced endometrial cancer who had disease progression in the 3 months prior to entry (Table 2) [65]. Seven (37%) of the first 19 patients achieved a clinical benefit response, defined as a complete or partial response or prolonged stable disease (at least 16 weeks), thus allowing expansion to the second stage. Of the 35 patients whose demographic data were
available, 23 had an endometrioid carcinoma, five had a carcinosarcoma, six had a UPSC and one patient had a clear cell carcinoma. Thirty-four patients had prior chemotherapy including doxorubicin, taxanes or platinum, and 14 had prior pelvic irradiation. Nine (33%) out of 27 patients evaluable for response, had a clinical benefit response, and eight of these nine patients had endometrioid carcinoma. The most common side-effects were fatigue, anemia, mouth sores and nausea/vomiting. According to these results, ridaforolimus is well tolerated and shows encouraging activity in patients with endometrioid-type endometrial carcinoma.

A randomized phase II clinical study conducted by Oza et al. (2011) compared ridaforolimus compared with progestin or chemotherapy in female adult patients with advanced endometrial carcinoma [66]. The study demonstrated that ridaforolimus significantly prolonged progression-free survival in women with advanced endometrial cancer compared with control treatment progestin or chemotherapy, and demonstrated the promise of ridaforolimus as a novel treatment. The safety profile was consistent with prior ridaforolimus experience [66]. Further clinical trials should be undertaken in combination settings with hormonal and cytotoxic agents.

Multiple other agents which target the PI3K/AKT/mTOR pathway are currently being developed and may eventually warrant investigation in patients with endometrial cancer. Some of these agents include bisindolylmaleimide MKC-1 (formerly known as Ro-31-7453), an orally active, small molecule that reduces phosphorylated AKT; and Suberoylanilide Hydroxamic Acid (SAHA), which was demonstrated to decrease expression of mTOR [67]. However, the clinical efficacy of such agents is yet to be explored.

**Tyrosine kinase inhibitors**

Tyrosine kinase receptors such as EGFR, VEGFR, FGFR and IGFR are good targets for anticancer therapies. Tyrosine kinase inhibitors inhibit the activation of tyrosine kinase receptors which subsequently suppress the phosphorylation of Mitogen Activated Protein Kinases (MAPK), CDK 1, PI3K/Akt, RAF 1 and Rb-1, which lead to cell proliferation, survival, motility, and adhesion (Figure 3). The detailed characteristics of these inhibitors of tyrosine kinase receptors have been discussed below.

![Figure 3: Simplified schematic illustration of the EGFR, FGFR and VEGFR pathway.](image)

**Egfr inhibitors**

The Epidermal Growth Factor (EGF) receptor (EGFR) family i.e. ErbB family serves as an excellent candidate for therapeutic intervention based on studies of tumor formation,
which is defined by aberrant cell proliferation [68,69]. The EGFR family consists of four distinct tyrosine kinase cell surface receptors i.e. EGFR (ErbB1 or HER-1), HER-2/neu (ErbB2), HER-3 (ErbB3) and HER-4 (ErbB4) that are expressed in the normal endometrium and may be overexpressed in endometrial cancers. The epidermal growth factor receptors are activated by binding to EGF-like growth factor [36,39]. Downstream serine/threonine kinase cascades are then induced, leading to cell proliferation and the inhibition of apoptosis (Figure 3). Increased expression of EGF-related protein and EGFR may contribute to a drug resistant phenotype. Inhibition of EGFR with monoclonal antibodies leads to growth arrest, and a similar and potentially synergistic effect is anticipated with inhibition of EGFR tyrosine kinase activity. EGFR is commonly expressed in normal endometrium, but its overexpression in endometrial cancer is associated with advanced stage and poor prognosis [70]. In patients with type I EC, the EGFR expression has been reported to occur in 46% of cases whereas in patients with type II EC, 34% of cases are reported to show EGFR expression [70]. Although the clinical significance of EGFR has not been studied well in EC, it may have a dual role. EGFR overexpression did not affect disease progression in type I EC, although it affects disease progression in type II EC. EGFR overexpression in type II EC is associated with high grade and adverse clinical outcome [70]. Therefore, EGFR targeted therapy may be clinically active as adjuvant therapy in well-defined subgroups of type II EC patients with EGFR and ErbB-2 overexpression. Two major classes of ErbB-targeted therapies i.e. ‘ErbB-specific Tyrosine Kinase Inhibitors (TKIs)’ and ‘Monoclonal Antibodies (MoAbs)’ have been developed as described further.

**Erbb-specific tyrosine kinase inhibitors (tkis)**

Antagonists to EGFR include small molecule inhibitors such as gefitinib (Iressa, ZD1839), erlotinib (OSI-774), lapatinib (GW-572016) and imatinib (Gleevec; STI-571) (Table 4).

<table>
<thead>
<tr>
<th>SL no.</th>
<th>Agent</th>
<th>Structure</th>
<th>Class of EGFR inhibitor</th>
<th>Type of therapy</th>
<th>Phase of clinical trial</th>
<th>Source</th>
<th>Mechanism of action</th>
<th>Adverse effects</th>
<th>References</th>
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<tbody>
<tr>
<td>1</td>
<td>Gefitinib</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>Reversible EGFR inhibitor</td>
<td>Persistent or Recurrent</td>
<td>Phase II Endometrial Cancer</td>
<td>Astra Zeneca</td>
<td>inhibits EGFR tyrosine kinase by binding to the ATP-binding site of the enzyme.</td>
<td>Rash, diarrhea, nausea, vomiting</td>
<td>Leslie, 2009 [71]</td>
</tr>
<tr>
<td>2</td>
<td>Erlotinib</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>Reversible EGFR inhibitor</td>
<td>Locally Advanced and/or Metastatic Endometrial Cancer</td>
<td>Phase II</td>
<td>Genentech /Roche</td>
<td>inhibits EGFR tyrosine kinase by binding to the ATP-binding site of the enzyme.</td>
<td>Rash, diarrhea, nausea, fatigue, headache</td>
<td>Oza, 2008 [72]</td>
</tr>
<tr>
<td>3</td>
<td>Lapatinib</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>Irreversible Dual TKI for EGFR/HER2</td>
<td>Recurrent or Persistent Endometrial Cancer</td>
<td>Phase II</td>
<td>Glaxo Smith Kline</td>
<td>inhibitor of the intracellular tyrosine kinase domains of both EGFR and HER-2 receptors</td>
<td>Rash, diarrhea, nausea, vomiting</td>
<td>Konecny, 2008 [73]</td>
</tr>
<tr>
<td>4</td>
<td>Imatinib Mesylate</td>
<td>Irreversible TK Inhibitor</td>
<td>Recurrent or Persistent Uterine Carcinoma sarcoma</td>
<td>Phase II</td>
<td>Pfizer</td>
<td>covalently binds to the ATP binding site of the intracellular kinase domain</td>
<td>neutropenia, thrombocytopenia, anemia, headache, edema, nausea, rash, and musculoskeletal pain</td>
<td>Huh, 2010 [74]</td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>5</td>
<td>Cetuximab</td>
<td>Chimeric monoclonal antibody, an EGFR inhibitor</td>
<td>Progressive or Recurrent Endometrial Cancer</td>
<td>Phase II</td>
<td>Chimeric (mouse/human)</td>
<td>Binds to the EGFR</td>
<td>Fatigue, nausea, constipation, headache, pain, and vomiting</td>
<td>Bansal, 2009; [34] Slowmovitz, 2010 [75]</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>trastuzumab</td>
<td>Monoclonal antibody, HER2/neu receptor inhibitor</td>
<td>With Stage III, Stage IV, or Recurrent Endometrial Cancer</td>
<td>Phase II</td>
<td>Humanized (from mouse)</td>
<td>binds to the domain IV of the extracellular segment of the HER2/neu receptor</td>
<td>Cardiac dysfunction</td>
<td>Fleming, 2007; [76] Fleming, 2009; [77] Vandenput, 2009 [78]</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4:** Small Molecule Tyrosine kinase inhibitor for endometrial cancer treatment.

**Gefitinib (ZD1839)**

Gefitinib [4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline] is a low-molecular-weight (447 KD) synthetic compound, an orally active, potent, and reversible inhibitor of EGFR tyrosine kinase [79-81]. Gefitinib inhibits the autophosphorylation of EGF-stimulated EGFR in a variety of EGFR-expressing human cancer cell lines. This compound is less active against HER-2/neu kinase; inhibition of non-EGFR stimulated cell growth requires a 40-fold higher concentration of gefitinib. At higher concentrations, gefitinib inhibits EGFR2-HER-2/neu and other intracellular transmembrane tyrosine kinase receptors, such as Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), Platelet-Derived Growth Factor (PDGF), and Insulin-Like Growth Factor (IGF) [79,82]. Interestingly, some investigators have speculated that co-expression of HER-2/neu with EGFR may increase tumor sensitivity to gefitinib, and it is believed that the EGFR/HER-2/neu heterodimer may be the most active heterodimer within the EGFR family [83].

Gefitinib has now been used in a therapeutic trial for women with advanced endometrial cancer (Gynecologic Oncology Group Study 229C). Therefore, it is important to investigate how these types of endometrial cancers respond to such therapy. Type 1 tumors have high EGF expression as a result of estrogen-induced EGF gene transcription [84]. If EGF levels are high, such tumors may respond to EGFR blockade even with normal cellular levels of EGFR. Therefore, the ligand, the receptor, or both may be over-expressed leading to inappropriate cellular proliferation, but in any of these circumstances Gefitinib may be an effective therapy. However, type II endometrial tumors may be more resistant to EGFR-targeted therapies, at least in part, because they lack a genomic response that includes the modulation of the gene products described above in type I-derived endometrial cancer cells and thus have lower expression of EGFR. In addition, the response of the more aggressive type II tumors may be limited by the constitutive activation of other signaling pathways [85].

Single-agent gefitinib has been assessed in a phase II trial on advanced endometrial cancer, and the preliminary analysis of 29 patients revealed that one (3%) patient experienced a complete response and several others had stable disease at 6 months (Table 4) [71]. The treatment was well tolerated but adverse effects were seen in the gefitinib arm when compared with the placebo arm. Hence, further studies are required to evaluate its efficacy. In order to improve the antitumor efficacy, gefitinib may be combined with chemotherapy or radiotherapy. Gefitinib administered with chemotherapeutic agents is reported to demonstrate supra-additive tumor inhibition compared to either treatment alone in other cancer types [79].
Erlotinib (OSI-774)

Erlotinib ([6,7-bis (2-methoxy-ethoxy)-quinazolin-4-yl]-(3-ethylphenyl)-amine) is a novel low-molecular-weight quinazolin derivative that acts as a selective, potent and reversible inhibitor of EGFR tyrosine kinase activity [86-89]. Like gefitinib, erlotinib does not decrease the level of EGFR protein [90].

In phase II or III clinical trials, erlotinib has shown anti-tumor activity in several malignancies including lung, ovarian, head and neck, and biliary tract cancers [91-93]. However, the use of erlotinib in women with recurrent and metastatic endometrial cancer was not promising in a phase II dose escalation study, with only 1 partial response among 27 women (Table 4) [72]. One probable reason seemed to be the lack of EGFR mutations in responders or correlation of response with gene amplification in cases of endometrial cancer. These studies suggested that there is need to develop sensitive techniques to assess the expression level of EGFR in tumors before the formulation of personalized therapy for a patient.

Lapatinib (GW572016)

Lapatinib (lapatinib ditosylate), is an orally active dual inhibitor of the tyrosine kinase domain of both EGFR and HER-2 [94,95]. Lapatinib has shown activity in a number of different metastatic and advanced tumor cell lines which overexpress either EGFR or HER-2, and has recently shown positive results in clinical testing as well. The studies demonstrated that enhanced expression of EGFR ligands such as amphiregulin, TGF-a, epiregulin, or NRG1 or HER3 may be associated with sensitivity to lapatinib in endometrial cancer [96,97]. Lapatinib exerts growth inhibition in a PTEN-independent manner. In contrast, lapatinib-resistant cell lines exhibited high Androgen Receptor (AR) levels or Epithelial-To-Mesenchymal Transition (post-EMT) features. In a recent study, the effect of Lapatinib was explored in human endometrial cancer cell lines where a wide range of clinically achievable drug concentrations, additive and synergistic interactions were observed for lapatinib plus carboplatin, paclitaxel, docetaxel, and doxorubicin. These observations provide a clear biologic rational to test lapatinib as a single agent or in combination with chemotherapy in endometrial cancer with HER2 overexpression. Expression of EGFR, its ligands, HER3, AR, and post-EMT markers warrant further evaluation to help define patients with HER2-non-overexpressing endometrial cancer (Table 4) [73]. Lapatinib showed preliminary activity in endometrial cancer. A phase II study of GOG trial of lapatinib has been closed and results are awaited in patients with recurrent or persistent endometrial adenocarcinoma (www.clinicaltrials.gov) [98].

Imatinib (STI 571)

Imatinib (Gleevec), a small molecule antagonist, has been shown to specifically inhibit c-Kit, Abl (Abelson proto-oncogene), and Platelet-Derived Growth Factor Receptor (PDGFR) and intermediates of downstream cascades e.g. Akt [99]. It has been shown to be an effective treatment for patients with chronic myelogenous leukemia and gastrointestinal stromal tumors. These cancers are characterized by activating mutations of the Abl and c-Kit tyrosine kinases, respectively. Their ligands are growth factors and hormones like epidermal growth factor, platelet derived growth factor and insulin. Imatinib furthermore showed synergistic effects with the established chemotherapeutic substances in leukemia and in adenocarcinomas [100,101]. All these observations led to the consideration, that Imatinib could be useful for the treatment of a wider range of solid cancers [102,103].

Slomovitz and collaborators reported the preliminary data of a phase I trial aimed to determine the maximum tolerated dose and the dose limiting toxicity of escalated doses of imatinib daily in association with paclitaxel with stage III/IV or recurrent UPSC. Eight patients were evaluable for efficacy [42]. One of the two patients with measurable disease had a partial response, and two of the six patients with no measurable disease had recurrence...
after 5 and 10 months, respectively (Table 4). Further clinical trial studies are warranted to better evaluate the efficacy of this combination treatment [74].

**Anti-erbb monoclonal antibodies (moabs)**

The anti-ErbB monoclonal antibodies include trastuzumab and cetuximab (Table 4).

**Cetuximab/C225**

Cetuximab (IMC-C225; Im-Clone Systems, New York, NY) is the first human-mouse chimeric monoclonal antibody derived from the murine anti-EGFR monoclonal antibody M225 [104]. Cetuximab has shown growth inhibitory effects in EGFR overexpressing cell lines and tumor xenografts [105]. Irreversible binding of EGFR by Cetuximab facilitates receptor internalization and subsequent degradation. Mechanistically, binding of Cetuximab arrest the progression of cell cycle at the G1 phase, upregulates the expression of p27Kip1, and subsequently inhibits tumor growth and metastasis [105-108]. Cetuximab may also work by mediating complement fixation and antibody dependent- cell mediated cytotoxicity. More importantly, this antibody is able to block the activation of the tyrosine kinase domain of the EGFR following stimulation with a specific ligand [109]. Cetuximab has also been reported to inhibit the production of pro-angiogenic factors such as vascular endothelial growth factor, interleukin-8, and basic fibroblast growth factor [110].

Molecular-targeted therapy with cetuximab for endometrial cancer could be advantageous for patients with advanced or recurrent endometrial cancer [34,111]. A phase II clinical study conducted by Slomovitz et al., [61] showed clinical benefit response rate of 17% [75]. Patients with recurrent endometrial cancer received an initial cetuximab dose of 400 mg/m² followed by weekly doses of 250 mg/m² for a 4 week cycle. All were treated until progression or toxicity. Rash was the most common adverse event at 72%, but only 1 patient had a grade 3 rash. Fatigue, nausea, constipation, headache, pain, and vomiting were other common side effects of treatment (Table 4). These results suggest that cetuximab can be beneficial for endometrial cancer patients over-expressing EGFR. Translational studies are under way to help identify which patients are most likely to respond to this therapy [75].

The majority of the studies with anti-EGFR agents merely required EGFR to be present in the tumor. Since, there are no accurate diagnostic methods of determining the level of EGFR expression in a tumor; the clinical benefits from anti-EGFR therapies are limited. More work is required to be done for the precise indication of EGFR status and also the predictive value of currently used preclinical models should be re-assessed with a view to improve the clinical outcome of EGFR targeting agents.

**Trastuzumab**

Trastuzumab (Herceptin; Genentech, South San Francisco, CA) is a monoclonal antibody (MAb) that targets extracellular domain of HER2. HER-2 amplification or overexpression has been demonstrated and linked to prognosis in endometrial cancer as well as in many other cancer types [112,113]. Although HER-2 overexpression observed in serous carcinoma of the uterus provides a strong biologic rationale for the use of trastuzumab in the treatment of this malignancy, a phase II study examining the use of trastuzumab in women with HER-2 positive endometrial cancer did not report any activity (Table 4) [76,78,114]. Another study evaluated efficacy of single agent transtuzumab aginst advanced or recurrent HER-2 positive endometrial carcinoma and explored predictors for HER2 amplification. Here again it did not demonstrate activity against endometrial carcinoma with HER2 overexpression although full planned accrual of women with HER2 amplified tumors was not achieved due to slow recruitment [115]. In other types of cancers trastuzumab was found to be more effective when used in combination with chemotherapy [116,117]. Therefore a combination therapy approach may be needed to demonstrate the benefit of trastuzumab in the treatment of HER2 positive endometrial cancer.
VEGFR Inhibitors

Angiogenesis clearly plays an important role in the pathogenesis of endometrial cancer. Vascular Endothelial Growth Factors (VEGF) and their receptors play a key role in tumour angiogenesis, and anti-angiogenic agents have been developed to target this pathway (Figure 3). VEGF expression has been found in 56-100% of endometrial cancer specimens, and has been correlated with high histological grade, deep myometrial invasion, angiolymphatic invasion, lymph vascular space involvement, lymph node metastasis and poor prognosis [118-120]. Therefore, targeted therapy specific to these markers is a rational approach to be incorporated into the treatment strategy for this disease. There have been relatively few clinical trials of antiangiogenesis therapy for endometrial cancer [121,122]. However, the few agents that show effective blockade of angiogenesis can produce responses in patients with advanced or recurrent uterine neoplasms.

Bevacizumab

Bevacizumab is a recombinant humanized monoclonal IgG1 antibody which neutralizes VEGF-A (the predominantly active species of VEGF) [123]. Although the mechanism of action of these antibodies is still under study, the anti-VEGF antibody bevacizumab has been approved for treatment of various solid cancers including colorectal, lung, and breast cancers, as well as glioblastoma, renal cell carcinoma and endometrial cancer [38,119,122].

A retrospective trial of bevacizumab in 11 women with recurrent uterine neoplasms (nine with epithelial endometrial carcinomas and two with leiomysarcomas) who received bevacizumab combination therapy resulted in two patients with partial responses, three patients with stable disease and five patients with progression of disease (one patient was unable to be assessed) [120]. The Gynecology Oncology Group has completed a phase II trial of single-agent bevacizumab in the treatment of metastatic/recurrent endometrial cancer (GOG 229-E). Fifty-six patients were enrolled. Fifty-two patients were eligible and evaluable. Median age was 62 years, and prior treatment consisted of one or two regimens in 33 (63.5%) and 19 (36.5%) patients, respectively. Twenty-nine patients (55.8%) received prior radiation. Adverse events were consistent with those expected with bevacizumab treatment. Seven patients (13.5%) experienced clinical responses (one complete response and six partial responses; median response duration, 6.0 months), and 21 patients (40.4%) survived progression free for at least 6 months. Median PFS and overall survival times were 4.2 and 10.5 months, respectively [124,125]. Cardiovascular side effects as well as pain were the most common toxicities, with each developing in 7.5% of the patients (Table 5). It was suggested that Bevacizumab is well tolerated and active based on progression-free survival at 6 months in recurrent or persistent endometrial cancer and warrants further investigation.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Pathway targeted/Type of therapy</th>
<th>Intervention/Agent</th>
<th>Phase of clinical trial</th>
<th>Health authority</th>
<th>Study</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Antiangiogenic agents</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recurrent or Persistent Endometrial Cancer</td>
<td>Bevacizumab</td>
<td>Phase II</td>
<td>United States: Food and Drug Administration</td>
<td>Active, not recruiting</td>
<td>Aghajanian, 2009; [124] Aghajanian, 2011 [125]</td>
</tr>
<tr>
<td></td>
<td>Advanced/recurrent uterine carcinoma (UCA) or carcinomas (CS)</td>
<td>Sorafenib tosylate</td>
<td>Phase II</td>
<td>United States: Food and Drug Administration</td>
<td>Active, not recruiting</td>
<td>Nimeiri, 2010 [126]</td>
</tr>
</tbody>
</table>
Table 5: Other inhibition for endometrial cancer treatment.

Sorafenib (BAY 43-9006; NEXAVAR)

An alternative strategy to antibodies targeting VEGF signalling is via inhibition of the VEGF-receptor tyrosine kinases with small molecules. These agents have the advantage of being orally available, and will ultimately be a more cost-effective treatment than the biological therapeutics.

Sorafenib is a potent, orally administered, multitargeted tyrosine kinase inhibitor with antiproliferative and antiangiogenic activities, has been recently studied in advanced, recurrent endometrial cancer and carcinosarcomas [126]. Sorafenib was originally described as an inhibitor of B- and c-RAF kinase, but also has activity against several receptor tyrosine kinases, including vascular endothelial growth factor receptor 2 (VEGFR2), Platelet-Derived Growth Factor Receptor (PDGFR), FLT3, Ret and c-Kit [43]. A recent phase II trial in patients with advanced/recurrent endometrial cancer has shown that sorafenib may result in clinical benefit for a limited number of endometrial cancer patients [35]. Preliminary results have shown that pharmacological inhibition of B-RAF by sorafenib sensitised endometrial cancer cells to TRAIL-induced apoptosis, by down-regulating FLIP. Among 39 patients with endometrial carcinomas, 5% had a partial response and 50% had stable disease after 2 months of therapy (Table 5). These preliminary results were not encouraging.

Sunitinib

The orally bioavailable sunitinib blocks the tyrosine kinase activities of Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2), PDGFR-b), and c-kit, thereby inhibiting angiogenesis and cell proliferation. This agent also inhibits the phosphorylation of Fms-related tyrosine kinase-3 (FLT-3), another receptor tyrosine kinase expressed by some leukemic cells [127]. Sunitinib showed preliminary activity in endometrial cancer. This trial will proceed to a second stage of accrual to further explore the efficacy and safety of sunitinib in advanced endometrial cancer [126,77].
Fibroblast growth factor receptor 2 (fgfr2) inhibitors

The recent identification of activating mutations in FGFR2 in endometrial tumors has generated a new avenue for the development of targeted therapeutic agents [130,131]. Fibroblast Growth Factor Receptor 2 (FGFR2) is regulated on the basis of the balance of FGFs, heparan-sulfate proteoglycans, FGFR2 isoforms, endogenous inhibitors, and microRNAs [132]. The altered FGFR2 gene causes the receptors to become active, leading to cell proliferation. In an analysis of 116 primary endometrioid endometrial cancers, FGFR2 and K-ras mutations were mutually exclusive. FGFR2 mutations, on the other hand, were seen concomitantly with PTEN abrogation [133]. Molecular silencing of FGFR2 or treatment with PD173074 resulted in cell cycle arrest and induction of cell death in endometrial cancer cells with FGFR2-activating mutations. The targeting of cancers with altered FGFR2, if successful, may form a part of the future personalized medicine. In vitro studies have shown that endometrial cancer cell lines with activating FGFR2 mutations are selectively sensitive to a pan- FGFR inhibitor, PD173074, both enhanced cell cycle arrest and induced cell death [127,133]. Oral administration of AZD2171 or Ki23057 inhibits in vivo proliferation of cancer cells with aberrant FGFR2 activation in rodent therapeutic models [132]. Several agents with activity against FGFRs are currently in clinical trials. Among PD173074, SU5402, and AZD2171 functioning as FGFR inhibitors, AZD2171 is the most promising anticancer drug [127]. Investigation of these agents in Endometrial Cancer Patients with Activating FGFR2 mutations is warranted [131].

Folate receptor alpha inhibition

Folate Receptor (FR) is overexpressed in several epithelial malignancies, especially in gynecological cancers, such as breast cancer, ovarian cancer, and endometrial cancer [134,135]. FR-α targeted therapy in high-risk endometrial carcinomas has been studied and found to be overexpressed in a significant proportion of endometrial adenocarcinoma, especially in the high-grade, high-stage tumors that are most likely to relapse, therefore making it an attractive therapeutic target [136-138]. An evaluation of a large cohort of high-risk endometrial cancer specimens found an association between FR-α expression and adverse outcome [135]. In vitro studies have also shown that tamoxifen upregulates FR-α expression; thus, estrogen negative cancers may also benefit from FR-α targeted therapy [139]. A number of FRα-targeted Thymidylate Synthase (TS) inhibitors (raltitrexed, pemetrexed, nolatrexed, ZD9331 and GS7904L) for the imaging and treatment of cancer have entered clinical studies in the last few years [140].

Microtubule stabilization

Microtubules are major dynamic and structural cellular components important in several cell functions, including cell division, cell signaling, and intracellular trafficking. Thus, tubulin and microtubules are compelling cellular targets for chemotherapy, as these functions are often dysregulated in many types of cancer. In fact, among anticancer agents, those that target microtubules constitute one of the most effective classes of chemotherapeutics for survival prolongation in advanced disease [141]. The list of compounds, which bind to tubulin or microtubules, is large and consists of chemically unique compounds that bind to the microtubule polymer and stabilize microtubules, such as the taxanes (Taxol and Taxotere) and the epothilones. Drugs that bind at the taxane site of tubulin are used extensively for the treatment of a wide variety of human cancers, including ovarian, endometrial, prostate, and breast cancers [142-147]. Among new class of tubulin polymerization agents, epothilones and ixabepilone have undergone the clinical trials for endometrial cancer therapy. However the outcomes of these trials are still awaited.

Epithilones and ixabepilone

The epothilones are macrolide antibiotics obtained from the fermentation of the
mycobacterium *Sorangium cellulosum*. The epothilones are a novel class of microtubule-stabilizing agents that show preclinical activity in taxane-resistant settings [38,129]. Like the taxanes, these compounds promote tumor cell death by tubule polymerization, arresting cell cycle progression at the G2/M phase, and inducing apoptosis [148]. However, unlike taxanes, epothilones demonstrated low susceptibility to multiple mechanisms of tumor cell resistance including multidrug resistance, βIII-tubulin overexpression, and β-tubulin mutations [149-151]. These observations led to the preclinical and clinical evaluation of a wide range of synthetic and semi-synthetic analogs of these agents, with ixabepilone emerging as a promising agent.

Ixabepilone (BMS-2474550) is a semisynthetic lactam derivative of epothilone B and is part of this new class of cytotoxic tubulin polymerization agents [152,153]. The documented activity of ixabepilone in breast cancer and other solid tumors refractory to taxanes prompted a clinical trial with this agent in patients with recurrent or persistent endometrial carcinoma who had failed in prior chemotherapy regimen [128,154]. A phase I trial of ixabepilone reported stable and minimal responses in patients with advanced ovarian and endometrial cancers [155]. A phase II study of ixabepilone in patients with recurrent or persistent endometrial adenocarcinoma is closed and results showed that the overall response rate was 12%. Ixabepilone was administered as a 40 mg/m² infusion over 3 h. Out of the 50 patients that were enrolled in the trial, one patient achieved a complete remission, while five others achieved partial remission lasting between 4.2 and 19.8 months. Thirty patients (60%) experienced stable disease for at least 8 weeks and the median progression-free survival was 2.9 months, while 20% patients had a progression-free survival of at least 6-months. The median overall survival was 8.7 months [146,154]. The major side effects were neutropenia and leucopenia, seen in 52% and 48% of patients respectively. Based on these results, Ixabepilone appeared to be quite active, particularly in a chorot who had received prior paclitaxel. Two studies are now recruiting to look this agent in endometrial cancer. The first is a randomized Phase III study comparing ixabepilone with paclitaxel or doxorubicin in women with locally advanced, recurrent or metastatic endometrial cancer as second-line chemotherapy (ClinicalTrials.gov) [156]. This study represents the first trial aiming for FDA approval as a second-line treatment in this disease. The second is a randomized, Phase II trial of three different cytotoxic/biologic combinations as a first line treatment in patients with advanced-stage or recurrent endometrial cancer. This study is a three arm which compares combinations of two of the standard first line agents (carboplatin and paclitaxel) while doxorubicin is replaced by bevacuzumab or tensirolimus. In the third treatment arm, carboplatin remains in addition to bevacuzumab but the paclitaxel is replaced by Ixabepilone. (ClinicalTrials.gov) [157] although results are awaited of this study and follow-up trials, ixabepilone may appear as a future first-line agent in the treatment of endometrial cancer.

**Histone deacetylase inhibitors (hdacis)**

Epigenetic alterations are believed to be involved in the repression of tumor suppressor genes and promotion of tumorigenesis in endometrial cancer; novel compounds endowed with a Histone Deacetylase (HDAC) inhibitory activity are an attractive therapeutic approach. Histone Deacetylase Inhibitors (HDACIs) were able to mediate inhibition of cell growth, cell cycle arrest, apoptosis, and the expression of genes related to the malignant phenotype in a variety of endometrial cancer cell lines [158,159]. For example, HDACI have been shown to induce differentiation of endometrial carcinoma cell lines, which resemble normal endometrial epithelial cells in the absence of ovarian steroid hormones. Furthermore, HDACIs were able to induce the accumulation of acetylated histones in the chromatin of the p21WAF1 gene in human endometrial carcinoma cells. Moreover, some studies have shown that HDACI had an important growth inhibitory effect on endometrial cancer cell lines, by decreasing the proportion of cells in S phase, and increasing the proportion of cells in the G0-G1 and or G2-M phases of the cell cycle [158]. There was also up-regulation of
p21, p27 and E-cadherin, and down-regulation of Bcl-2 and cyclin-D1 and -D2. The growth-suppressor effects seem to be irrespective of the p53 gene status [160-165]. In xenograft models, some HDACIs have demonstrated antitumor activity with only few side effects. The classes of HDACIs that have been identified are: (a) organic hydroxamic acids (e.g., Trichostatin A (TSA) and Suberoylanilide Bishydroxamine (SAHA)) (b) short-chain fatty acids (e.g., butyrates and valproic acid (VPA)), (c) benzamides (e.g., MS-275), (d) cyclic tetrapeptides (e.g., trapoxin), and (e) sulfonamide anilides (Table 6) [166].

<table>
<thead>
<tr>
<th>Substance groups</th>
<th>Derivatives</th>
<th>Isotype**</th>
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<td>Hydroxamates</td>
<td>Trichostatin A (TSA)*</td>
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<tr>
<td></td>
<td>Suberoylanilide Hydroxamic Acid (SAHA, vorinostat)*</td>
<td>I,II</td>
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<tr>
<td></td>
<td>LBH589 (panobinostat)</td>
<td>I,II</td>
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<td>PCI24781 (CRA-024781)</td>
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<td>LAQ824</td>
<td>I,II,IV</td>
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<td></td>
<td>PXD101 (belinostat)</td>
<td>I,II,IV</td>
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<td>I,II,IV</td>
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<td>m-Carboxycinnamic Acid Bishydroxamide (CBHA)*</td>
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<td>Short chain fatty acids</td>
<td>Butyrate</td>
<td>I,IIa</td>
</tr>
<tr>
<td></td>
<td>Valproate</td>
<td>I,IIa</td>
</tr>
<tr>
<td></td>
<td>AN-9</td>
<td>I,IIa</td>
</tr>
<tr>
<td></td>
<td>OSU-HDAC42</td>
<td>I,IIa</td>
</tr>
<tr>
<td>Benzamides</td>
<td>MS-275 (entinostat)*</td>
<td>1,2,3,9</td>
</tr>
<tr>
<td></td>
<td>MGCD0103*</td>
<td>1,2,3,11</td>
</tr>
<tr>
<td></td>
<td>Pimelic diphenylamide</td>
<td>1,2,3</td>
</tr>
<tr>
<td></td>
<td>M344*</td>
<td>1,2,3</td>
</tr>
<tr>
<td></td>
<td>N-acetyldinaline (CI-994)</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Cyclic tetrapeptides</td>
<td>Apicidine*</td>
<td>I,II</td>
</tr>
<tr>
<td></td>
<td>Trapoxins</td>
<td>I,II</td>
</tr>
<tr>
<td></td>
<td>HC-toxin</td>
<td>I,II</td>
</tr>
<tr>
<td></td>
<td>Chlamydacin</td>
<td>I,II</td>
</tr>
<tr>
<td></td>
<td>Depsipeptide (FR901228 or FK228) (romidepsin)***</td>
<td>1,2,4,6</td>
</tr>
<tr>
<td>Sulfonamide anilides</td>
<td>N-2-aminophenyl-3-[4-(4-methylbenzenesulfonylamo)-phenyl]-2-propenamide</td>
<td>1,2,3,9,11,16</td>
</tr>
<tr>
<td>Others</td>
<td>Depudecin</td>
<td>NDH-51</td>
</tr>
<tr>
<td></td>
<td>KD5150</td>
<td>KD5150</td>
</tr>
</tbody>
</table>

*Inhibitors have been studied using endometrial cancer cell lines Ishikawa, HEC-1B, HEC5, RL95, KLE, AN3CA and Ark2**Class I: HDAC1, -2, -3 and -8; class IIa: HDAC4, -5, -7, and -9; class IIb: HDAC 6, and -10; class III: SIRT1-7; class IV: HDAC11.

Table 6: Overview of histone deacetylase inhibitors used for clinical and/or research purposes in various cancer types.

A variety of structurally distinct classes of compounds that inhibit deacetylation of both histone and non-histone proteins have been identified. Despite the shared capacity of each class of HDACIs to promote histone acetylation, individual HDACIs exert different actions on signal transduction and the induction of differentiation and/or apoptosis. Many questions are currently still unanswered with respect to HDACI specificities for definite tumor subtypes and the molecular mechanisms underlying HDACI-induced differentiation, cell cycle arrest and apoptosis. In addition, the regulation mechanisms of the specific gene expression and recruitment of HDAC complex to the specific promoter sites remain still to be determined. Also, it is yet unclear to what extent different HDACs exhibit different and potentially overlapping functions, and it is important to distinguish the HDAC specificity of HDACIs for the development of selective therapy on the molecular level [167]. It is required...
to improve our understanding of why transformed cells are more susceptible to the effect of HDACIs than normal cells. Also, combinations of HDACIs with differentiation-inducing agents, with cytotoxic agents, and even with gene therapy may represent novel therapeutic strategies and new hope for the treatment of endometrial cancer.

**Claudins**

Epithelial receptors for Clostridium Perfringens Enterotoxin (CPE), also known as claudins (encoding the tight-junction proteins), may well prove to be the next target therapy for endometrial cancer, especially against aggressive disease variants. It has been shown that UPSC overexpress claudins-1,-3 and -4, while clear-cell ones overexpress claudins -3, and -4 [168-171]. A dose-dependent cytotoxic effect was demonstrated when primary and metastatic UPSC cell lines were incubated with different concentrations of CPE in vitro. Moreover, multiple intra-tumoral injections of well-tolerated doses of CPE led to massive tumor necrosis and inhibited subcutaneous tumor growth in UPSC xenografts expressing claudin-3 and claudin-4 from severely combined immunodeficient mouse. Intraperitoneal injections of sublethal doses of CPE had a significant inhibitory effect on tumor progression, with extended survival of animals with chemoresistant UPSC in the abdominal cavity. Because claudin-3 and/or claudin-4 may be expressed in some normal human tissues, such as gut, lung and kidney, the potential high toxicity of CPE at doses used for systemic cancer therapy in animal models might limit its use in humans to local/regional treatments [169,172]. More recently, study on pancreatic and ovarian cancer showed that monoclonal antibody therapy against claudin-4 appeared to be promising [173]. Therefore, as a novel therapeutic approach for endometrial cancer, there is a need to identify and develop specific agents against claudins with minimal cross reactivity in normal tissues.

**Summary and Future Implications**

Endometrial cancer is the most common gynaecological cancer in western countries. The standard treatment consists of surgery alone, or in combination with radiation, chemotherapy and/or hormonal therapy [174]. For women with disease progression, chemotherapy is the only currently available treatment option, and limited benefit has been seen in such cases, emphasizing the need for new therapies.

In the past few years medical and gynecological oncologists have started to investigate novel molecular targeted agents capable of inhibiting cellular signaling mechanisms that control endometrial cancer cell proliferation and survival. Consequently, new therapies have been proposed targeting specific molecular alterations such as the mTOR inhibitors and the tyrosine kinase inhibitors. The mTOR pathway can be activated by several mechanisms including activation of PI3k, tyrosine kinase receptor and ras. Moreover, loss of function of p53 may also result in the activation of mTOR. The preliminary data from phase II studies on mTOR inhibitors CCI-799 and AP-23573 show promising results in the treatment of heavily pre-treated patients with this malignancy. ErbB-2 overexpression in UPSC supports clinical investigations on the use of trastuzumab in chemoresistant patients. Clinical evidence also suggests that it may be possible to improve on the activity achieved by these agents when they are given in combination with existing cytotoxic agents or with each other. The development of agents that target variety of pathways holds great promise for the treatment of endometrial cancer. Evidence to this is the Phase II GOG trial looking at new treatment combinations that involve carboplatin, paclitaxel, bevacizumab (a VEGF inhibitor), temsirolimus (an mTOR inhibitor) and the epothilone ixabepilone. Results of such trial may re-define first-line therapy for advanced endometrial cancer and are thus eagerly anticipated. Another approach may include multiple targeting agents, for example, dasatinib which inhibits a large array of targets including SRC family, BCR-ABL, C-KIT and PDGF. It also target EphA2 receptor, a member of the Ephrin family which is found to be over-expressed in high proportion of endometroid tumors and correlates with advanced disease and poor prognosis [175,176]. It is hoped that clinical trials of such agents for endometrial cancer might give optimistic outcome in future.
Continued investigation into the molecular pathways of endometrial cancer development and progression with the use of advanced array technology, will enhance existing knowledge of this disease process and will lead to the discovery of novel and druggable targets. Further, it is important to design trial that relies on demonstration of druggable target prior to establishment of given targeted therapies. As the results of the clinical trials discussed in this review become available along with further understanding of the tumorigenesis of endometrial cancer, it might be possible to better define an ideal treatment regimen and sequence for patients with advanced disease. However, with the experience of clinical trials conducted with various agents so far, the importance of appropriate patient selection cannot be ruled out. Therefore, for better success of trials there is a need for inclusion of biomarker-driven clinical trials in future trial designing.

Acknowledgement

The authors are greatful to Indian Council of Medical Research for financial support.

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Chapter-8

Herbal Treatment Strategies for Breast Cancer

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Abbreviations: BC: Breast Cancer; CAM: Complementary and Alternative Medicines; SEER: Surveillance, Epidemiology, and End Results; HRT: Hormonal Replacement Therapy; TCM: Traditional Chinese Medicine; CHP: Chinese Herbal Products; MCF: Michigan Cancer Foundation

Abstract

Complementary and alternative medicine (CAM) use is common amongst cancer patients particularly breast cancer patients are the most likely users. Numerous studies support that herbal medicines are amongst the most commonly used group of treatments among CAM. Herbal remedies are assumed by the general public to be safe, cause less complications and are less likely to cause dependency. The foremost reasons of CAM (including herbal remedy) popularity among breast cancer patients include improving quality of life, supporting conventional cancer treatment, preventing recurrence and eventually to prolong survival.

However there remains limited scientific evidence on the efficacy and safety of natural therapies including herbal remedies. The potential for interactions of herbal with conventional medications, consumption of high dose with consequent adverse effect and the limited, insufficient data concerning the possible hazards of herbal consumption, pose serious public health issues. Vigorous clinical trials are required to establish the efficacy or presence of any adverse effects of such preparations.

Introduction

Breast Cancer

Breast cancer (BC) continues to be the most frequently occurring cancer in women around the world. The increased incidence, mortality, economic costs, is a burden shared among women globally [1]. BC continues to be a major public health problem in developed as well as developing countries [2]. Unfortunately, in spite of improved diagnostic skills and breakthrough in effective treatment, BC continues to be the leading cause of cancer deaths among women worldwide, with approximately 375,000 deaths in the year 2000 [1,2]. Moreover it is also accountable for greater than one million of the estimated 10 million diagnosed cancers worldwide each year in both sexes [3]. One in ten of all new cancers diagnosed worldwide each year are a cancer of the female breast. The burden differs between countries and regions showing variations in incidence, mortality and survival rates [1,5].

Cancer Treatment Strategies
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than in developed countries (23.1 versus 63.2 per 100,000 women) however the incidence rates vary widely between and within countries [4,6]. Over the past several decades, the risk of breast cancer in developed countries has increased by one to two percent annually [7].

Researcher believe that these variations are related to multiple factors such as health habits, socio-economic status, lifestyle changes (for example, later childbearing and dietary changes), exposure to radiation or family history, associated changes in menstrual patterns in addition to access and availability of care, early detection, and access to the current knowledge regarding BC [5,8]. Age is the single most important risk factor for breast cancer. Compared with women in their twenties, women are 10 times as likely to develop breast cancer in their thirties, 40 times as likely in their forties, 60 times as likely in their fifties, and 90 times as likely after age 60 [7].

Prognosis is heavily dependent on stage of disease at presentation, however based on the Surveillance, Epidemiology, and End Results (SEER) registries in the US 5-year survival for localized cases in 1994 was about 97% but was only about 25% for cases with metastatic disease [3]. In developing countries, the differences in survival by stage at diagnosis are also very marked.

Female breast cancer incidence rates vary nearly five-fold across the regions of the world. In 2008, rates ranged from around 20 per 100,000 in Eastern and Middle Africa to 90 per 100,000 in Western Europe [9]. The countries with the highest incidence rates in 2008 were Belgium and Denmark (109 and 101 per 100,000, respectively) while the incidence rate of UK women was high at 11th highest out of 184 countries worldwide [9]. Taking example of the US, breast cancer is the second most common cause of cancer death among women ages 40 to 49 years with an estimated 40,410 breast cancer deaths in US in year 2005 [10]. Breast cancer death rates for women in the US are higher than those for any other cancers, besides lung cancer. The chances that breast cancer will be responsible for a woman’s death is about 1 in 36 (about 3%) [3]. However based on the stage of diagnosis, breast cancer is treated with a multidisciplinary approach involving surgery, radiation and medical oncology including chemotherapy or hormonal therapy. Doctors today most commonly employ a combination of local treatments that remove or destroy cancer in the breast (such as surgery and radiation) and systemic treatments that destroy or control cancer cells throughout the body (such as chemotherapy and hormonal therapy).

**Complementary and alternative medicines (CAM)**

Several new studies have discovered that most patients on cancer therapy are concurrently self-medicating with one or several complementary and alternative medicines (CAM) [11]. CAM is the term used for medical products and practices that are not part of standard medical care. Standard care is practiced by medical doctors and allied health professionals, such as nurses and physical therapists. “Complementary medicine” refers to treatments that are used with standard treatment such as is using acupuncture to help with side effects of cancer treatment. “Alternative medicine” refers to treatments that are used instead of standard treatment for example using a special diet to treat cancer instead of a method that a cancer specialist/doctor suggests. Standard treatments are based on scientific evidence from research studies, while CAM is based on claims made by CAM treatment providers. The consequence of most of these agents on the effectiveness and toxicity of regular anticancer treatment have not been considered, which poses a great threat to patients [11].

CAM is often associated with different terms such as “natural, holistic, home remedy or Eastern medicine” and is divided into five broad categories [12].

i) Mind-Body Medicines including hypnosis, yoga,

ii) Biologically based practices such as herbs, vitamins, special diets
iii) Manipulative or body based practices of massage, chiropractic care
iv) Energy medicine
v) Whole medical system

The prevalence of CAM (including herbal treatment) use is estimated at 25% among residents of the United Kingdom [13,14] 50% among German, French [14], and Australian [15] populations, and 42% to 69% among residents of the United States [16,17]. CAM is a major growth industry in Europe [13], and that trend is now mirrored in the United States, where typical characteristic of a CAM user is described as wealthy, white Americans with good education, commonly between the ages of 30 to 50 years, and residing in the northeastern or western regions of the country [13,18].

A study by Janette et al. (2013) supported above finding of CAM utilization by a large proportion of cancer patients (mean 36%, range up to 80%) [19] And among cancer patients, breast cancer patients remain as the most likely users [20]. Furthermore, among the CAM users almost half of the patients (50%) use herbal supplements or anti-oxidants at some phase during their cancer treatment. The maximum use of CAM among people with cancer is in women [21], particularly with breast cancer, who are of younger age, with higher levels of education, have more advanced disease and are of Asian ancestry [19-21]. Furthermore, the majority of patients do not notify their doctors about their CAM utilization [19,22].

A summary of 26 surveys across 13 countries concluded that the prevalence of CAM use by cancer patients overall was 31.4% (range, 7% to 64%). In the United States between 1990 and 1997, the prevalence of CAM use increased from 33.8% to 42.1%, and the number of visits to CAM practitioners increased from 427 million to 629 million visits with an estimated out-of-pocket expenditure of $34.4 billion in year 1997 [13].

**Herbal Treatment and Breast Cancer**

Among Complementary and alternative medicines, herbal medicine is the most commonly used group of treatment. Herbal treatment is the oldest used system of medicine in the world with more than 2000 years history [23]. Other names used for herbal therapy are phyto-medicine, phyto-therapy or botanical medicine. It is a medicine made exclusively from plants such as roots, bark, flowers, seeds, fruits, leaves, or branches and is used in all societies and common to numerous cultures including Asia, Africa, Europe and America. There are various types of herbal medicine that spring from different cultures around the world however they vary in the way they are prepared and in their treatment approaches [24].

Certain herbs defend the body from malignancy by augmenting detoxification or cleaning role of the body. Some biological response modifiers, derivatives of herbs, are recognized to hinder the growth of cancer by modifying the activity of precise hormones and enzymes, while other herbs diminish lethal side effects and complications of chemotherapy and radiotherapy [25]. Moreover, phytoconstituents resulting from the herbs such as Vinca rosea, Taxus species, Allium sativum, Aloe vera, Angelica sinensis, Astragals membranaceous, Glycine max, Glycyrrhiza glabra, Hordeum vulgare, Hydrocotyle asiatica, Medicago sativa, Morinda citrifolia, Panax pseudoginseng, Saussurea lappa, Taxus wallichiana, Tinospora cordifolia, Viscum album, Withania somnifera, Zingiber officinale etc. have been used in numerous preparations to improve function of the body’s immune cells that stimulates production of cytokines including interleukin, interferon, tumor necrosis factor as well as colony stimulating factor. These preparations assist the body to battle cancer more efficiently and also decrease the harmful side effects of chemotherapy and radiotherapy [25].

Herbal formulae have been prescribed to adults, children, and elderly, as well as pregnant and lactating mothers. Compared to Eastern part of the world where herbal treatments play a central role, they are not as popular in the United States. Such treatments form a
complete medical system that is integrated in modern hospitals and clinics throughout most of Asia. Literature documents the current popularity of natural alternatives to conventional medical treatments, especially among patients with chronic life-threatening diseases such as cancer [26].

Most cancer patients combine CAM (herbal remedies) with conventional therapy in the hope of boosting the effect of conventional medicine [22,27]. A study of women being treated for early stage breast cancer showed that 10.6% had been using one or more CAM at the time of diagnosis, while an additional 28.1% began using CAM (including herbal remedies) after surgery [11]. Similarly a multinational survey found that 35.9% of cancer patients were either past or present users of complementary and alternative medicine. Herbal medicines were by far the most commonly used group of treatments, escalating in use from 5.3% before the diagnosis of cancer to 13.9% after the diagnosis of cancer [24].

Generally, herbal products are utilized for two reasons, first, to lessen symptoms of disease and second to prevent sickness. Examples include palliative use of St. John’s Wort (Hypericum perforatum) for relief of acute depression, the use of Ginkgo biloba for enhancement in perception/understanding and the use of Echinacea for improving cold symptoms [28]. In the second circumstance, herbal supplements are taken especially in the anticipation of averting disease or modifying the effects of threat for certain illnesses. Such as intake of green tea and other flavonoid rich botanicals to yield benefit of the natural antioxidants in them and the consumption of garlic because of the high organo-sulfur compounds that have been experimentally proven to prevent cancer in animals [28].

In the domain of cancer prevention, herbs may performance through numerous mechanisms to shield the body. Initiation of phase I and phase II metabolic enzymes by herbal supplements is quite typical and maybe liable for some of this action [28]. These phase I and II enzymes provide major protection against carcinogenesis, mutagenesis, and other forms of toxicity mediated by carcinogens through initiation of their metabolism, particularly phase 2 enzymes such as glutathione S-transferases (GSTs), UDP-glucuronosyl transferases, and quinone reductases [29]. Taking example of garlic, its intake and supplement use is prevalent in both, Eastern and Western cultures [30]. Garlic along with numerous other organo-sulfur compounds derived from garlic demonstrate robust chemo-preventive action against experimentally induced cancers of the mammary gland as well as esophagus, stomach, colon, liver and lungs [28]. Initiation of phase I and phase II enzymes, nonetheless, can result in a likely significant side effect of herbal products. Such as St. John’s Wort that is extensively utilized, has been shown to encourage the CYP3A family of activation enzymes, through which half of current medications are also metabolized, hence offering the likelihood of herb-drug interactions [28].

**Common Herbs used Globally in Treatment for Breast Cancer**

**Echinacea**

*Echinacea*, a member of the family *Asteraceae* is a wild herb that grows primarily in the Great Plains and eastern regions of North America. It is also cultivated in Europe. Three different species of the plant are used in herbal remedies namely *Echinacea purpurea*, *Echinacea angustifolia*, and *Echinacea pallida* however *Echinacea purpurea* is most frequently used for research and treatment. Other common names associated with Echinacea are purple coneflower, Kansas snakeroot and black Sampson [31]. Studies have found that *Echinacea purpurea* increases the amount of natural killer cells in the experimental mice and suggested that *E. purpurea* could be a possible treatment for anti-tumor therapy in the future [31]. Winston et al supported the ability of Echinacea, which is rich in flavonoids that act as an immune-stimulant, by promoting the activity of lymphocytes, increasing phagocytosis by
macrophages and the activity of natural killer cells and inducing interferon production [32] at the same time reduces the adverse effect of radiotherapy and chemotherapy. It has also been used in an effort to prolong survival time in patients with advanced stage cancer. Commercial preparations of echinacea juice have been shown to increase cytokine production by macrophages [33]. A series of in-vitro studies have demonstrated that Echinacea stimulates various immune cells including Echinacea purpurea macrophages, polymorphonuclear granulocytes and natural killer cells. Effects on T-cell and B-cell activation and proliferation are less clear. Several constituents of Echinacea are considered to play a role in its effects on the immune system [34].

**Licorice**

Glycyrrhizin is a chief constituent of licorice root and is a sweet tasting triterpenoid saponin. Licorice is a perennial plant that grows in southern Europe, Asia, and the Mediterranean. The dried roots and underground stems of the plant are used in herbal remedies. Glycyrrhizin along with its aglycone and glycyrrhetinic acid have also been stated to encourage activity of interferon, supplement the movement of natural killer cells and modulate the growth response of lymphocytes through augmentation of IL-2 production [32,35-37]. Experimental studies have recognized number of substances in licorice that may help avert DNA mutations, reduce tumor development, or even destroy cancer cells including breast cancer, prostate cancer, and leukemia cells. In studies with mice, glycyrrhizin and glycyrrhizic acid decreased the initiation of colon, liver, uterine, and breast cancers [32]. Licorice root also contains powerful antioxidants, as well as certain phyto-estrogens that can perform some of the functions of the body’s natural estrogens. Research has demonstrated that this estrogenic effects of licorice components help to slow the progression of breast cancer [38,39]. Other studies have further shown that the bioactive compounds of licorice may be chemopreventive in other cancers, including prostate cancer.

**Cat’s Claw**

Two species of cat’s claw, *Uncaria guianensis* and *U. tomentosa*, found in northern regions of South America and belonging to madder family (Rubiaceae), have also gained much attention particularly in the West because of their immune-stimulant properties as they contain a rich source of phytochemicals with more than 30 known constituents including at least 17 alkaloids, along with glycosides, tannins, flavonoids, sterol fractions, and other compounds found in the root and stalk bark [32,40].

This indigenous herb has led scientists in the US and other countries to closely examine its effects in the body. Their findings suggest that this botanical agent exerts powerful anti-inflammatory and antioxidant effects that support DNA repair, joint health, immune function, and normal cell division [41].

Cat’s claw extract inhibits the production of tumor necrosis factor-alpha, an inflammatory messenger that sets the stage for both acute and chronic inflammation. Likewise it inhibits the activation of nuclear factor-kappa beta, an inflammatory “switch” that is associated with cancer and other deadly diseases [42]. Extracts and fractions of cat’s claw have been reported to stimulate T cells, macrophages, and other components of the immune system [32] as well as have antimutagenic and antiinflammatory properties [32,40].

**Garlic**

(*Allium sativum*) intake of garlic as a therapeutic agent has been practiced since past several decades to treat numerous illnesses. It encompasses hundred or more biologically beneficial secondary metabolites, such as alliin, alliinase, allicin etc. Garlic oil comprises of an amino acid identified as Alliin, which is transformed to Allicin once its bulbs are
crushed. Allicin is a predecessor to numerous sulphur comprising compounds that are accountable for the taste, aroma as well as its pharmacological properties. Ajoene, another sulphur holding compound, present in garlic oil, impedes mutagenesis while selenium act as a cellular antioxidant. Researches have also discovered the existence of bioflavonoids cyanidin and quercetin, which are liable for antioxidant characteristic of garlic [43-45].

The antitumor property of Garlic is attributed to its high level of a wide-ranging diversity of organic sulfides and polysulfide’s. It is known to augment action of the immune system by activating lymphocytes and macrophages to kill cancer cells. It is also identified to interrupt the metabolism of tumor cells [32].

Moreover, garlic prevents creation as well as development of cancer by increasing action of the natural killer cells and the macrophages. Researchers have discovered that garlic enhances amount of the suppressor T cells and turns the lymphocytes further cytotoxic to tumor cells. It also restrains metastases by averting union or adhesion of the circulating tumor cells to the blood vessels. The ripened extract of garlic shields DNA from the harmful influence of carcinogens, surges activity of detoxifying enzymes, hustles up elimination of chemical carcinogens and boost body’s immune system. Further, (mature garlic extract) it is known to prevent development of several tumors including those of the breast, lungs, stomach, colon and bladder. An investigation done at the National Medical Centre and Hospital in Japan has shown that the Garlic extract lessens complications of radiotherapy and chemotherapy as well [25,46,47].

**Flaxseed**

*(Linum usitatissimum)* contains a rich supply of lignans. By bacterial fermentation these plant lignans are transformed into mammalian lignans (enterolactone and enterodiol) in the colon and they can thereafter behave as estrogens [32,48]. Mammalian lignans appear to be anti-carcinogenic because lignan metabolites hold a structural resemblance to estrogens and can attach to estrogen receptors to hinder the development of estrogen-stimulated breast cancer. In women with breast cancer urinary excretion of lignans is reduced, while the intake of flaxseed powder enhances urinary concentration of lignans by many folds [32,49-52].

Experimental studies of flaxseed diet on a mouse model, have demonstrated dose dependent inhibition of breast tumor growth [53]. Human trials also confirmed similar beneficial effects. A double-blinded, randomized controlled trial of dietary flaxseed demonstrated dramatic protection with a significant apoptosis (tumor cell death) and reduced cell proliferation [54]. Likewise women eating more flaxseeds were found to have a 42% reduced risk of death from postmenopausal breast cancer and a dramatic (40 percent) reduction in all causes of death [55]. Another interesting study on flax followed women for up to 10 years and found a 51% reduced risk of all-cause mortality and a 71% reduced risk of breast cancer mortality.

**Turmeric**

*(Curcuma longa)* imparts a rich yellow color to food. The root and rootstock, or rhizome, of the plant contains curcumin, which is considered to be the active ingredient. Its anti-mutagenic action as well as cancer inhibition activity is attributed to its phenolic constituents. Turmeric has been shown to curb the progress of breast, lung, stomach and skin malignancies [32,56]. Its antioxidant curcumin (a diferuloylmethane), has been shown to be a successful anti-inflammatory agent in humans and slows down the development of cancer by averting the production of toxic eicosanoid such as PGE-2 [32]. This anticancer outcome has been established in all the phases of tumor growth, i.e. initiation, promotion and progression. Studies have revealed that Curcuma longa inhibits production of nitrosamine that enhances natural antioxidant functions of the body. Curcuma longa increases levels of glu-
tathione and other non-protein sulphahydryls and acts directly on several enzymes [25,57]. Numerous research also advocates that curcumin hampers the initiation of cancer as well as encourages its deterioration [25,32]. Laboratory studies support that curcumin interferes with several important molecular pathways involved in cancer development, growth, and spread while researchers report that curcumin inhibits the formation of cancer causing enzymes in rodents [58].

**Burdock**

*(Arctium lappa)* is a root that is found in Europe and Asia. It has many medicinal qualities and has been used in many herbal remedies. The root is sweet to taste and has a gummy consistency. Traditionally, burdock has been used as a remedy for measles, arthritis, tonsillitis, while in modern times; burdock is used in oncology as well as in many other serious health problems. It comprises of some powerful anticancer features that averts mutations in the oncogenes. It has been used in the management of breast cancer, ovary, bladder, malignant melanoma, lymphoma and cancers of the pancreas. Studies document that it decreases the size of tumor, ease the pain and prolongs the survival phase [25].

During the development of tumors, very large amounts of nutrients (oxygen and nutrients) are required to sustain the rapid proliferation of tumor cells. However, tumor cells can still survive under extreme conditions such as low oxygen and low carbohydrate availability due to their relatively high tolerance to hostile environment. Arctigenin, an active compound found in the seeds of burdock, has the ability to eradicate nutrient-deprived cancer cells [59]. In addition flavonoid-type antioxidants and some other active polyphenol antioxidants found in the root of burdock may account for the suppressive effects on cancer metastasis [60]. It has been shown that extracts of the root protect cells from toxic substances and lower the mutations of cells.

Tannin, a phenolic compound, is one of the most common active compounds found in the root of burdock. It induces macrophage responses, inhibits tumor growth and possesses immuno-modulatory properties [61].

**Carotenoids**

Carotenoids are the pigments found in green, leafy herbs, rose hips, and the herbs used as coloring agents, such as paprika, saffron, and annatto. Epidemiologically, vegetable and fruit consumption has constantly been associated with a reduced incidence of a variety of cancers [62] and dietary carotenoid intake from these sources has similarly been correlated with a reduced cancer risk [63]. Major carotenoids with antioxidant activity that have been extensively evaluated with regard to their cancer chemopreventive ability include β-carotenes, β-cryptoxanthin, lycopene, lutein and zeaxanthin. However, studies tend to agree that overall intake of carotenoids is more protective than a high intake of a single carotenoid [64].

The carotenoid pigments are powerful antioxidants and exhibits several biological activities, including the scavenging of free radicals, shielding against oxidative injury to cells, enhancement of gap junctions, immunomodulation and regulation of the enzyme activity involved in carcinogenesis as well as stimulate the immune function of the body [65]. Freudenheim et al. have shown that the intake of carotenoid-rich foods, specifically vegetables, as well as lutein and zeaxanthin, is significantly associated with a lower risk of developing premenopausal breast cancer [66]. People who have elevated serum levels of carotenoids demonstrate less risk of both cancer and heart diseases [32,67].

**Green tea**

Polyphenolics in green tea (*Camellia sinensis*) are also recognized to have anti-mutagenic
and anti-cancer actions. The most abundant polyphenol in green tea is EGCG (epigallocatechin-3-gallate) which has also been the focus of pre-clinical and clinical research in a variety of health settings. EGCG have substantial free radical scavenging activity and protect cells from DNA damage caused by reactive oxygen species [68]. Tea polyphenols have also been shown to inhibit tumor cell proliferation and induce apoptosis in laboratory and animal studies [69] while in other studies, tea catechins have shown to inhibit angiogenesis and tumor cell invasiveness as well as modulate immune system function [70]. Some evidence from animal studies suggests that tea has a protective effect against stomach and colon cancers and the threat of cancer in number of organs is diminished by utilization of green and black tea or their primary catechins [71]. Furthermore studies have also found that green tea as well shields the body from harmful effects of radiation [25,32]. Although many of the potential beneficial effects of tea have been attributed to the strong antioxidant activity of tea polyphenols, the precise mechanism by which tea might help prevent cancer has not been established [69].

Ginseng

Panax ginseng is a perennial plant grown in China, Korea, Japan and Russia. The dried roots of the plants are used in traditional medicines to treat a variety of conditions, including cancer. Constituents of ginseng have been shown to inhibit the production of tumor necrosis factor in mouse skin, [72] inhibit the growth and proliferation of cancer cells in animal models, inhibit cell proliferation, induce differentiation, and stimulate interferon levels. Other tumor cell processes may also be interfered with by ginseng constituents [73].

Research investigations conducted in Korea recommended that ginseng may decrease the threat of malignancies in humans [32]. Most effective form of ginseng is known to be its extract and powder compared to fresh sliced ginseng, ginseng juice, or ginseng tea, for diminishing the threat of cancer. In a large-scale, case-control study in Korea, researchers observed that the incidence of human cancer decreased steadily with duration of ginseng use and total lifetime use of ginseng [32,74]. Other studies report that ginseng hinders cancer development by disrupting with the DNA synthesis. Panax ginseng contains several active constituents which helps restart the natural killer cells that are injured during chemotherapy and radiotherapy, stimulate the macrophages and encourages formation of antibodies [25].

Black cohosh

Black cohosh is among the most frequently cited agent being used by breast cancer patients during their radiotherapy and chemotherapy. Black cohosh (Cimicifuga racemosa) is a shrub like plant commonly seen in the eastern forests of North America [11]. It has been used since centuries by Native American herbalist for health issues like menopausal symptoms, pre-menstrual discomfort and dysmenorrheas, as well as to induce abortion and numerous other problems. The herb was a chief component of the once famous patent medicine Lydia Pinkham’s Vegetable Compound and was also listed in the 19th century Pharmacopoeia. Drug stores offers a range of black cohosh preparations, supported by the recommendations of herbalists and traditional healers as being safe, effective and natural therapy for menopausal symptoms [11]. Black cohosh is being utilized by women who have been recommended to avoid HRT (Hormonal Replacement Therapy) by their doctors, who are at high risk for breast cancer or who have discontinued HRT after a diagnosis of breast cancer [11].

A thorough and systematic scientific literature on black cohosh is amazingly sparse. Majority of studies have revolved around the herb’s effects on menopausal symptoms [75]. The active component(s) have not been definitively identified; triterpene glycosides (including
27 deoxyactein, acetin, and cimifugoside), have been assumed to be the vital constituent, but resins and caffeic, isofurulic and fukinolic acid also have been put forward as to having biological actions [11]. It is ambiguous whether the herb has estrogenic or anti-estrogenic activities with numerous studies in the literature posing considerable debate over the subject [11,76]. Research discloses only a small number of studies testing the effects of black cohosh on breast cancer cells with contradictory conclusions, few reporting increase and others no change or decrease in the development of breast cancer cells in culture [11].

Sara et al. cautioned that black cohosh should not be considered to be a harmless herb that is insignificant to the health of cancer patients or to the outcome of conventional cancer therapy.

Unless the outcome of black cohosh is vividly clear, the utilization of this and similar herbal product by breast cancer patients must be discouraged [11]. While another study by Einbond et al. indicated that relatively low concentrations of acetin or the ethyl acetate, fraction of black cohosh, can cause synergistic inhibition of human breast cancer cell proliferation when combined with different classes of chemotherapy agents [77].

**Traditional Chinese medicine (TCM)**

Another form of treatment considered part of CAM is Traditional Chinese medicine (TCM) this originated during ancient China and has evolved over thousands of years. It is based on Chinese medical principles primarily the energetic qualities of the herbs rather than the chemical properties as understood by Western pharmacology [78]. TCM has been gaining immense attention as well as acceptance and has presented a central platform to health care in many countries. In Taiwan, Chinese herbal products (CHP) have been an imperative part of health care for past several decades [79] and are fully compensated under the current National Health Insurance (NHI) system. There are over 2,000 different kinds of herbs (of which about 400 are commonly used).

Past clinical trials have established that Jia-wei-xiao-yao-san (Augmented Rambling Powder), which is the principle and most commonly approved method for managing breast cancer in Taiwan, may be an effective treatment for diminishing psychological (anxiety and depression) signs and symptoms in postmenopausal women [80,81]. Among the best, principally agreed method for managing breast malignancies, Gui-pi-tang (Ginseng and Longan Combination), Tian-wang-bu-xin-dan (Ginseng and Zizyphus Combination), and Suan-zaoren-tang (Zizyphus Combination), all of which have an extensive record of utilization, are believed to nurture the blood and soothe the nerves and are quite commonly prescribed by TCM doctors to relieve or minimize sleep disorder [82]. Other frequently approved methods are often for alleviating gastrointestinal distress (Ban-xia-xie-xin-tang, or Pinellia Combination), reduced appetite (Xiang-sha-liu-jun-zi-tang, or Vladimiria and Amomum Combination), exhaustion, weakness (Bu-zhong-yi-qi-tang, or Ginseng and Astragalus Combination), palpitation (Ren-shen-yang-rong-tang, or Ginseng Nutritive Combination), or enlargement or inflammation of lymph nodes (San-zhong-kui-jian-tang, or Forsythia and Laminaria Combination) [80].

However it is evident from Lai et al. study that TCM doctors in Taiwan recommend herbal remedies primarily for decreasing psychosocial sufferings and symptomatic distress [82]. Nonetheless, it is yet to be explained whether commonly prescribed CHPs comprising of ren shen (Panax ginseng-radix) and dang qui (Angelica sinensis-radix) for tumor management are anticipated by TCM doctors to reduce the treatment coupled toxicity or to improve the cancer resulting illness/symptoms. Further studies are necessary to evaluate such preparations and its efficacy as an add-on treatment for women undergoing conventional breast cancer management [82].
Anti-breast cancer agents discovered from Chinese herbal medicine

A number of anti-breast cancer agents have been discovered from Chinese herbal medicines (CHM), although some of the mechanisms of action have still not been clarified. The findings from some of the studies are summarized below;

**Alkaloids**: Alkaloids exist widely in CHM and natural products. It has been used in the treatment for many diseases with a long history. The anti-breast cancer activity of more than 20 alkaloids isolated from CHM has been investigated in vitro by determining the inhibitory activity against growth of human mammary cancer cell line BCAP [83]. Yang et al. reported that berbamine and camptothecin demonstrated significant inhibition for the growth of human mammary cancer cell line BCAP cell while Rescinnamine and tomatidine were found to have weak inhibition [83,84]. The study supported the potential in these ingredients as leading compounds for anti-tumor drugs in the future [84].

**Coumarins**: About forty coumarin compounds isolated from the traditional CHM have been screened for their antitumor activity, It was found that bergapten, cndilin, dicoumarol and notoptol exhibited weak inhibition for the growth of BCAP cell line. The inhibitory effects of psoralen and quercetin on the proliferation of human breast cancer cell lines MCF-7 were found to be able to inhibit proliferation [84].

**Flavanoids, and Polyphenols**: Franek and Zhou et al. reported baicalin (antipyretic) Flavins scutellarin (a circulatory stimulant) and two extracts from salvia miltiorrhiza (SM-470, circulatory stimulant) and camellia sinensis (Cam-300, antipyretic), inhibited the proliferation of the human breast cancer cell lines MCF-7 and T-47D, with baicalin being the most potent inhibitor [85]. Moreover, the combination of these compounds from different botanical classes offers enhanced therapeutic Benefits such as the combination of SM-470 (circulatory stimulant) with scutellarin, cam300 or baicalin, augmented the inhibition of cell proliferation [84].

Eulalia et al. reported Resveratrol (RES), a chemo-preventive molecule, to be able to inhibit the proliferation of tumor cells of different etiologies [86]. The study showed that RES altered the cell cycle and induced apoptosis in MCF-7 breast tumor cells by interfering with the estrogen receptor (ER)-dependent phosphoinositide 3-kinase (PI3K) pathway. Curcumin was also found to inhibit the proliferation as well as induced apoptosis of MCF-7 cells [84,86].

**Terpenoids**: The medicinal herb feverfew (Tanacetum parthenium), has been used as a folk remedy for the treatment of migraine and arthritis for long time [87]. Parthenolide, a sesquiterpene lactone, is considered as the primary bioactive compound in feverfew. Wu et al. measured the inhibitory activity of parthenolide against two human breast cancer cell lines (Hs605T and MCF-7) and one human cervical cancer cell line (SiHa) [84,87]. Among the tested constituents of feverfew, parthenolide demonstrated the highest inhibitory effect [87].

Zhang et al. studied the inhibitory effects of Ursolic acid (UA), apentacyclic triterpene acid on MCF-7 cell apoptosis [88]. The results showed that twenty-four hours after UA treatment, apoptotic cells increased dose dependently and the morphology changes of MCF-7 cells displayed many hallmark features of apoptosis, including chromatin aggregation and fragmented nuclei [84,88].

**Quinone and other chemical classes**: Plumbago zeylanica Linn. (Plumbaginaceae) has been used for treatment of some tumor diseases in Chinese herbal medicines with long history, of which plumbagin [89] is one of very important bioactive components. Liu et al also observed the anti-tumor activity of plumbagin in vitro. The results of which showed that plumbagin had significant cytotoxic effect however this promising result needs further exploration [84,89].
Another research study documented the mechanism of action and the effects of Artemisinin and its analog artesunate on the proliferation of human breast cancer MCF-7 cell line. However, artemisinin had weaker effect on the proliferation of MCF-7 cell, while artesunate effectively inhibited the proliferation of MCF-7 with higher apoptosis in vitro [84].

**Dandelion**: or *Taraxacum officinale* (TO), have a history of use in Chinese, Arabian and Native American traditional medicine, to treat a variety of diseases including cancer. To date, however, very few studies have been reported on the anti-carcinogenic activity of TO. Sophia Sigstedt et al. 2008 in her study investigated on three aqueous extracts prepared from the mature leaves, flowers and roots of Taraxacum officinale and its effect on tumor progression, proliferation and invasion [90]. It concluded that the crude extract of dandelion leaf decreased the growth of (Michigan cancer foundation) MCF-7/AZ breast cancer cells, whereas the aqueous extracts of dandelion flower and root had no effect on the growth [90].

**Vinca rosea**: (*Catharanthus roseus*) comprises of vinca alkaloids, which were the first phyto-constituents ever used to treat cancer. Research has led to the discovery of more than 70 alkaloids, which include vinblastine, vincristine (leurocrystine), alstonine, ajmalicine and reserpine etc. Vincristine is frequently administered in combination with other anticancer medications to treat cancers of the breast, lung, bladder and the cervix [25,91].

**Ochrosia elliptica**: Ellipticine is one of the simplest naturally occurring alkaloids from the leaves of the evergreen tree Ochrosia elliptica, which behaves as a powerful anticancer agent. Ellipticine and its offshoots are used to treat cancers of the breast and the kidney. Lipophilic derivatives of ellipticine operates by binding to the DNA [25,92].

**Herbal Remedies: Adverse Effect and Drug Interaction**

Unlike conventional drugs, herbal products are not tested with the scientific rigor required of conventional drugs, nor are they regulated for purity and potency [93]. Thus, some of the adverse effects and drug interactions reported for herbal products could be caused by impurities (e.g., allergens, pollen and spores) or variability in preparation. In addition, the potency of an herbal product may increase the possibility of adverse effects [93].

Taking example of Echinacea, although it is relatively safe, researchers cautioned that it may cause liver damage or suppress the immune system if used for more than 8 weeks. According to their recommendation, people taking medications known to cause liver toxicity, such as anabolic steroids, amiodarone (a drug for heart rhythm problems), and the chemotherapy drugs methotrexate and ketoconazole, should avoid echinacea use [33].

Similarly, cat’s claw lowers blood pressure, causes sleepiness, and diarrhea. People who are taking blood pressure medicines, blood-thinning medications, hormones, or insulin should be cautioned of possible drug-herb interaction (American cancer society) ginseng with increased heart rate, nausea, headaches. Since ginseng may have steroid/hormone like effects, some health professionals show concern against its use particularly in women who have had breast or endometrial cancer [25]. Flaxseed on the other hand is associated with bowel obstruction and bleeding disorder. Burdock interacts with anticoagulant/anti-platelet drugs, slowing blood clotting and increasing the chances of bruising and bleeding [25].

**Conclusion/Way Forward**

There has been a recovery of attention and interest, both scientifically and in terms of recognition, in the consumption of natural approaches in treatment of chronic diseases. Science has long accepted the importance of natural substances, such as digitalis, aspirin, penicillin, insulin, steroids, etc. Experimentations have shown that herbal drugs can play anticancer role by stimulating apoptosis and differentiation, augmenting the immune system, hindering angiogenesis and reversing multidrug resistance. Nevertheless, the mecha-
nism of the anticancer function has not yet been completely illuminated. Given that, several herbal medicines have not been tested comprehensively or even undergone basic research, often there is limited, insufficient data concerning the possible hazards and benefits of their consumption. In spite of the accepted perception of herbs as being safe, improves survival and quality of life, yet, a range of unfavorable events related with their consumption alone or in combination with standard conventional cancer treatment has been documented including the incidence of severe bone marrow depression.

Further research is needed to explore the molecular mechanism of herbal drugs including carefully controlled trials, establishing which constituents are effective, which will offer precious evidence for investigating and developing anticancer drugs in the future. A combined research and clinical program of integrative medicine with the medical and public health communities coming together to become more involved in this dialogue is needed.

<table>
<thead>
<tr>
<th>Categories</th>
<th>Active Agents</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Berbamine</td>
<td>Anti-mammary cancer</td>
</tr>
<tr>
<td></td>
<td>Camptothecin</td>
<td>Anti-mammary cancer</td>
</tr>
<tr>
<td></td>
<td>Rescinnamine</td>
<td>Anti-mammary cancer</td>
</tr>
<tr>
<td></td>
<td>Tomatidine</td>
<td>Anti-mammary cancer</td>
</tr>
<tr>
<td>Coumarins</td>
<td>Bergapten</td>
<td>Anti-breast cancer</td>
</tr>
<tr>
<td></td>
<td>Cnidilin</td>
<td>Anti-breast cancer</td>
</tr>
<tr>
<td></td>
<td>Dicoumarol</td>
<td>Anti-breast cancer</td>
</tr>
<tr>
<td></td>
<td>Notoptol</td>
<td>Anti-breast cancer</td>
</tr>
<tr>
<td></td>
<td>Psoralen</td>
<td>Estrogen-like activity</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>Estrogen-like activity</td>
</tr>
<tr>
<td>Flavonoids and polyphenols</td>
<td>Baicalin</td>
<td>Anti-breast cancer</td>
</tr>
<tr>
<td></td>
<td>Scutellarin</td>
<td>Anti-breast cancer</td>
</tr>
<tr>
<td></td>
<td>Resveratrol</td>
<td>Inhibiting PI3K pathway</td>
</tr>
<tr>
<td></td>
<td>Curcumin</td>
<td>Inhibit transcript of VEGFR and b-FGF</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Parthenolide</td>
<td>Anti-breast, anti-cervical cancer</td>
</tr>
<tr>
<td></td>
<td>Ursolic acid</td>
<td>Anti-breast cancer</td>
</tr>
<tr>
<td>Quinone and other chemical classes</td>
<td>Plumbagin</td>
<td>Anti-breast cancer</td>
</tr>
<tr>
<td></td>
<td>Artemisinin</td>
<td>Anti-breast cancer</td>
</tr>
<tr>
<td></td>
<td>Artemisinunate</td>
<td>Anti-breast cancer</td>
</tr>
</tbody>
</table>

**Table 1**: The Anti-Breast Cancer Agents and Its Effects


**References**


Abstract

The use of functional foods and nutraceuticals promotes the quality and length of life in patients suffering with cancer. Examples of nutraceuticals of interest includes antioxidant vitamins like vitamin-C & E, carotenoids improves the efficacy of cancer therapy by improving immune functions, increasing tumour response to radiation or chemotherapy, decreasing toxicity to normal cells. Healthy diet is very important in today’s lifestyle because good food can protect health, strengthen immune system, fight off cancer and other diseases. Moreover today’s lifestyle choices such as smoking, drinking, a lack of exercise and an unhealthy diet are a great start to an anticancer lifestyle. Diet has a powerful effect on our health. The best diet should include variety of vegetables, fruits and whole grains as they enhance the quality and length of life. The role of nutraceuticals in new era of 21st century showed enormous awareness and interest because of their presumed safety and potential nutritional and therapeutic effects.

Keywords: Antioxidant; Carotenoids and Chemotherapy; Immune System; Nutraceuticals

Introduction

Cancer chemoprevention is currently one of the most urgent projects in public health. According to epidemiological surveys, the majority of human cancers are related to two main factors; diet and smoking [1,2]. However, in general population, dairy consumption of certain foods has also been shown to have anticancer effects. This highlights the importance of environmental factors such as diet in cancer chemoprevention [1]. It is also evident that an understanding of the mechanisms of carcinogenesis is essential for cancer chemoprevention [1,2].

Cancer chemoprevention is defined as the use of specific natural and synthetic chemical agents to reverse or suppress carcinogenesis and prevent the development of invasive cancers. There has been an enormous growing awareness in recent years that dietary non-nutrient compounds can have important effects as chemopreventive agents, and considerable work on the cancer chemopreventive effects of such compounds in animal models has been undertaken [3]. Medicinal plants having nutritional values & physiological
effects in our life are a good source of food and medicines as they help in preventing variety of human ailments. The anticancer compounds from the plants have been found to be clinically active against various types of cancer cells because of the presence of potent anticancer substances. Recently, a greater emphasis has been investigated that diets rich in phytochemicals can reduce cancer [4,5]. The use of herbal remedies and dietary supplements are widespread throughout the world for the treatment of different types of cancers.

The main strategy for cancer in humans includes surgery, radiation and drugs. According to World Health Organisation (WHO) about three quarters of the world population relies upon the natural source mainly herbs for the treatment. The synthetic anticancer remedies are beyond the reach of common man because of cost factor [6]. The best dietary recommendations for the prevention of cancer should be plant derived diet which includes mainly vegetables, fruits and whole grains in order to get good health status and to postpone the development of diseases. The plant derived diet means food that comes from vegetables, fruits, nuts, grains and beans. Also as we know that foods are the main source of nutrients which mainly meet our nutritional requirement apart from essential nutrients for example proteins, carbohydrates, fat, minerals and vitamins in plant foods. Plant also produces non-nutrient chemical substances called phytochemicals means plant chemicals, which are the bioactive non-nutrient plant compounds present in fruits, vegetables, grains and other plant foods that have health related effects.

**Types of Diet**

- Plant rich diet
- Nutraceuticals & Functional foods diet
- Restrictive diet
- Macrobiotic diet
- Unsaturated fat diet
- Water Rich diet
- Immune boosting diet
- Non-fat dairy product diet
- Preserving process diet

**Plant rich diet**

The presence of plant rich diet means the presence of phytochemicals in vegetarian diet which might prevents the development and progression of diseases as plants are known rich source of antimitotic and antiangiogenic compounds. They have preventive effect on tumorigenesis and other chronic diseases.

Phytochemicals are large group of plant derived compounds found in fruits, vegetables, grains, beans, cereals, seeds and plant-based beverages such as tea and wine.

**Types of phytochemicals:** Based on their chemical structure phytochemicals are classified into different groups as:

**a. Phytochemicals**

i. Organo Sulfur compounds

ii. Alkaloids
iii. Nitrogen containing compounds

b. Flavonoids
i. Anthocyanins
ii. Flavones
iii. Flavanones
iv. Isoflavones
v. Flavonols
vi. Flavanols

c. Flavanols
i. Catechin
ii. Epicatechin
iii. Proanthocyanins

d. Proanthocyanins
i. Procyanidin
ii. Prodelphinidine

The consumption of a diet high in fruits and vegetables are associated with reduced risk of chronic disease [7].

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Example</th>
<th>Common Food Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonol</td>
<td>Quercetin</td>
<td>Apples, onions</td>
</tr>
<tr>
<td>Flavanol</td>
<td>Catechin</td>
<td>Tea, coffee, chocolate</td>
</tr>
<tr>
<td>Isoflavone</td>
<td>Genistin</td>
<td>Soy</td>
</tr>
<tr>
<td>Flavonone</td>
<td>Hesperidin</td>
<td>Grapefruit</td>
</tr>
<tr>
<td>Anthocyanidin</td>
<td>Cyanidin</td>
<td>Berries</td>
</tr>
<tr>
<td>Alkaloids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipomine A</td>
<td>Ipomoea batatas</td>
<td>sweet potato, tea, coffee</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Coffea arabica</td>
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</tr>
<tr>
<td>Theobromine</td>
<td>Thea sinensis</td>
<td>sweet potato, tea, coffee</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Thea sinensis</td>
<td>sweet potato, tea, coffee</td>
</tr>
<tr>
<td>Carotenoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β- carotene</td>
<td></td>
<td>mango, apricot, carrot, broccoli, spinach</td>
</tr>
<tr>
<td>Lutein</td>
<td></td>
<td>Egg yolk, red pepper, mustard</td>
</tr>
</tbody>
</table>

Table 1: Following are some of the examples of Phytochemicals & their food source.

Health benefits of phytochemicals

a. Isoflavones: (genistein & daidzein) present in soy beans, soy milk and tofu helps in reduction in blood pressure and increased vessel dilation [8].

b. Anthocyanin: in strawberries, red wine and blueberries helps in improvement of vision, inhibition of nitric oxide production, induction of apoptosis, decreased platelet aggregation and neuroprotective effects [9].

c. Proanthocyanidins & flavan-3-ols found in red wine, grape juice, grape extract, cocoa helps in inhibition of LDL oxidation, inhibition of cellular oxygenase and inhibition of
d. Sulfides, thiols: in garlic, onions, leeks, olive decreases LDL cholesterol.

e. Carotenoids: such as lycopene, beta-carotene found in carrots, tomatoes product and in various types of fruits and vegetables neutralizes the free radical that causes cell damage [10,11].

f. They are rich sources of important nutrient and by eating fruits and vegetables of different colours we can get wider range of phytochemicals for example [12,13].

<table>
<thead>
<tr>
<th>Colour</th>
<th>Examples of colourful foods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red</strong></td>
<td></td>
</tr>
<tr>
<td>Phytosome: Lycopene</td>
<td>Tomatoes, tomato product, pink grape, fruit and water melon</td>
</tr>
<tr>
<td>Red/Purple</td>
<td></td>
</tr>
<tr>
<td>Phytosome: anthocyanin</td>
<td>Grapes, grape juice, cranberries, Blackberries, strawberries, red apples &amp; red wine</td>
</tr>
<tr>
<td><strong>Orange</strong></td>
<td></td>
</tr>
<tr>
<td>Phytosome: carotenoids</td>
<td>Carrots, mangoes, apricots, pumpkin, sweet potatoes</td>
</tr>
<tr>
<td><strong>Orange/yellow</strong></td>
<td></td>
</tr>
<tr>
<td>Phytosome: ß-cryptothanxin</td>
<td>orange juice, oranges, peaches, papaya and nectarines</td>
</tr>
<tr>
<td><strong>Yellow/green</strong></td>
<td></td>
</tr>
<tr>
<td>Phytosome: lutein &amp; zeaxanthin</td>
<td>spinach, turnip, mustard, green, yellow corn, green pear, avocados &amp; honey dew melon</td>
</tr>
<tr>
<td><strong>Green</strong></td>
<td></td>
</tr>
<tr>
<td>Phytosome: indoles &amp; sulforphanes</td>
<td>Cabbage, Brussels, sprout &amp; kale</td>
</tr>
<tr>
<td><strong>White/Green</strong></td>
<td></td>
</tr>
<tr>
<td>Phytosome: allicin, quercetin &amp; flavonoids</td>
<td>onions, garlic, white grape juice &amp; white wine</td>
</tr>
</tbody>
</table>

Table 2: Following are some of the examples of colourful foods & their food source.

**Nutaceuticals & functional foods**

a. Nutaceuticals are natural and bioactive products which may be considered a food or part of a food and provides medical or health benefits including the prevention and treatment of disease.

Example of nutraceutical includes lutein, folic acid, cod liver oil capsules etc.

b. Functional foods are foods having one or more compounds with biochemical and physiological function provides the body with the required amount of vitamins, fats, proteins, carbohydrates necessary for healthy survival.

The most popular functional foods includes omega-3 eggs, omega-3 enriched yoghurts, calcium-enriched orange juice, Vitamin D, Multivitamin-minerals, selenium, coffee, chocolate, green tea, black tea, lycopene, soya meat, cheese, milk, blueberries, orange, legumes, yellow vegetables, turmeric roots, garlic, soya products, antioxidant vitamins (vitamin C & E, carotenoids).

c. Health benefits of nutraceuticals

1. Nutraceuticals have beneficial effects in cancer therapy and in diabetes [14].
2. They act as immune boosters.
3. Helps in chronic inflammation disorders
4. Helps in degenerative disorders
5. Nutraceuticals in the form of antioxidants, dietary fibers, omega-3 polyunsaturated
fatty acid (n-3 PUFAs), vitamins & minerals are recommended together with physical exercise for prevention and treatment of CVD [15].

6. Milk & eggs are the important animal sources of nutraceuticals like proteins & polyunsaturated fats or essential fatty acids (EFAs). EFAs are required for production and rebuilding of cells, to reduce blood pressure, lower cholesterol and triglycerides, reduce the risk of blood clots, helps prevent many diseases including arthritis, arrhythmias and other cardiovascular disease [16].

7. Lutein is one of the carotenoids and is found in many fruits and in vegetables includes mangoes, corn, sweet potatoes, carrots, squash, tomatoes as lutein is used in the treatment of visual disorders [17].

8. *Moringa oleifera* has an impressive range of medicinal uses with high nutritional value. The plant contains good source of protein, vitamins, β-carotene, amino acid and various phenolics [18].

9. They have a crucial role in the protection of numerous age related or chronic diseases.

10. Diet has an important role in the treatment of many diseases and the right choice of nutrients can help in disease prevention and improve the quality of life. Therefore nutraceuticals plays a crucial role in the protection of numerous age related or chronic disease [19].

d. Health benefits of functional foods

1. They have positive influence on health for example foods with added omega-3-fatty acids can help improve brain function in normal individuals [20].

2. Cholesterol lowering spreads have also been proven to be effective in human trials

3. Functional foods provide varying amounts of nutrients and energy to sustain growth or support vital processes [21].

4. They offer additional benefits that may reduce the risk of disease or promote optimal health for e.g. cranberry juice reduces urinary tract infection [22,23].

**Restrictive diet**

Restrictive diet is based on low calorie intake. Calorie restriction provides numerous secondary benefits, such as a greatly lowered risk for most degenerative conditions of aging & improved measures of general health. Calorie Restriction (CR) is also known as CRON, for Calorie Restriction with Optimal Nutrition.

In calorie restriction, energy intake is minimized, but sufficient quantities of vitamins, minerals & other important nutrients must be eaten.

Restrictive diet includes Ketogenic diet which eliminates all but non-starchy vegetable carbohydrates & replaces them with high amount of healthy fats and low to moderate amounts of high quality protein. Since cancer cells differ in that they cannot use fat (ketone) to survive-they need glucose & low oxygen environment.

It should include low carbohydrate, high fat foods like celery with cream cheese or a slice of cheese or nuts.

<table>
<thead>
<tr>
<th></th>
<th>Grams</th>
<th>Calories</th>
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<tbody>
<tr>
<td>Calorie</td>
<td></td>
<td>2,650</td>
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<tr>
<td>Fat</td>
<td>221</td>
<td>1967</td>
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<tr>
<td>Saturated fat</td>
<td>88</td>
<td>783</td>
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</tr>
<tr>
<td>---------------</td>
<td>----</td>
<td>-----</td>
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<tr>
<td>Polyunsaturated fat</td>
<td>12</td>
<td>103</td>
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</tr>
<tr>
<td>Monounsaturated fat</td>
<td>62</td>
<td>548</td>
<td>21%</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>24</td>
<td>87</td>
<td>3%</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>149</td>
<td>621</td>
<td>23%</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Diet chart plan for Restrictive diet (For Adults).

It is a dietary regimen in which subjects receive a reduced energy diet (typically, 20 to 40% reduction in total energy intake relative to an unrestricted comparison group). It provides beneficial effects on longevity & cancer risk in human beings [24,25].

**Health benefits of restrictive diet**

1. Restrictive diet provides fewer calories than normal diet and helps in preventing age-related problems and increases life span [26].
2. Restrictive diet improves recovery after a traumatic brain injury.
3. Due to age, the muscle mass and its function get lost but restrictive diet helps in preventing muscle preservation.
4. Reduced calorie diet combined with some physical activity helps in weight reduction.
5. Dietary modification improves overall health and the quality of life [24].

**Macrobiotic diet**

It is a dietary regimen and consists largely of whole grains, cereals, and cooked vegetables. Macrobiotics diets avoid the use of highly processed or refined foods. It emphasizes natural minimally processed foods and is compatible with many dietary recommendations not just for the prevention of cancer but also for the prevention of other chronic diseases [27-29].

Macrobiotic diet is associated with general health benefits and lower risk for several diseases. The standard macrobiotic diet consists of 50% to 60% organically grown fruits and vegetables and 5 to 10% soups made with vegetables, seaweeds, grain beans [30].

**A Macrobiotic practitioner plans diet by considering age, sex, where you live and exercise. It consists of:**

1. Organic whole grains such as brown rice, barley, oats and buckwheat- half of your food intake.
2. Locally grown, organic fruits & vegetables- up to a quarter of your food.
3. Soups made with vegetables, seaweeds, beans, lentils, peas & fermented soy- up to quarter of your food.

It also includes:

1. Cooking & storing food in pots & utensils made of wood, glass, stainless steel or china (ceramics)
2. Avoiding microwave ovens or cooking with electricity
3. Preparing food in a calm & peaceful environment. It may help in lowering the risk of getting heart disease, breast cancer and other cancers linked to high fat diet.
Unsaturated fat diet

Unsaturated fat diet is a healthy diet that helps in reducing heart diseases, lowers cholesterol levels. They are beneficial when they are replaced with saturated fats in the diet. Unsaturated fats are healthy type of fat that are liquid at room temperature. There are two types of unsaturated fats, which are known as

- Monounsaturated
- Polyunsaturated

Monounsaturated fats are liquid at room temperature but begin to solidify at cold temperature. They are found in olives, olive oil, nuts, peanut oil, canola oil and avocados. These types of fats lower LDL (bad) cholesterol & maintain HDL (good) cholesterol.

Polyunsaturated fats are also liquid at room temperature. These are found in sunflower, seasame, corn, cotton seed and soybean oil.

For example: Polyunsaturated Fatty Acid (PUFAs) present in fish oil, are part of human diet. Among PUFAs’s Docosahexaenoic Acid (DHA). DHA is having anti-inflammatory, antiproliferative, antiangiogenetic, antimetastatic properties [31].

Health benefits of macrobiotic diet:

1. Macrobiotic diet provides the body with essential nutrients so that it can function efficiently without loading it with toxins or excesses that must be eliminated or stored [32].

2. It helps in resulting freedom from fear and the new sense of control is two of the most important benefits of a macrobiotic practice.

3. A macrobiotic practice encourages the body’s natural ability to heal itself. If the body is not burdened by toxins and excesses, it can function better and thus heal any illness that does occur [33,34].

4. Macrobiotic approach requires a change in thinking from a static view of life to a dynamic and flexible one.

5. The main benefit of a standard macrobiotic diet is that the body becomes cleaner as toxins and old excesses are discharged [33].

Water rich diet

Water is our body’s principal chemical component and when our cells are not fully hydrated, they deteriorate and can't function at their peak level. This leads to the tissue damage which leads to aging. With age, the body naturally lose water and this water loss makes our body to heal, to scavenge for free radical and also to defend against invading bacteria and pathogens.

Moreover water regulates body temperature, eliminates toxins, carries nutrients and oxygen to the cells and provides moist environment for body tissues and joints.

For example:

1. Crisp lettuce: It contains 96% water and helps in keeping up with the hydration of the body.

2. Water Melon: It contains 91% water along with a good amount of vitamin A and C. It also contains lycopene, fibre and potassium.
3. Grape fruit: It contains 90% water. It is a good source of phytonutrients and vitamin C and provides energy and hydration.

4. Broccoli: It contains 89% water. It contains vitamin C, Ca+ fiber, iron and β-carotene which later get converted into vitamin A by body.

5. Low Fat Milk & yogurt: It contains 89% and 85% water content. It contains protein, phosphorus, potassium, vitamin A and D.

6. Coconut water: It contains 95% water and having low carbohydrate and low sodium content.

7. Cucumber: It contains 96% water

8. Strawberries contain 92% water and having 23% calories.

9. Healthy adult men need about 3.7 litre of water a day while women need about 2.7 litres [35].

**Health benefits of water rich diet:**

1. Drinking water helps maintain the balance of body fluids

2. Water Can Help Control Calories

3. It’s a diet rich in minerals, vitamins, antioxidants and fibre.

4. It keeps the body hydrated, preventing tiredness and fatigue.

5. It helps to flush waste and toxins out of the body. Water-rich foods are popular in detoxing and cleansing diets.

6. It reduces water retention.

7. It decreases the need for insulin in the body [36,37].

**Immune boosting diet**

The immune system is our body’s natural defence against harmful substances and abnormal cell development. Immune system contains different types of cells. Some examples of immune boosting food which prevent cancer include:

a. Broccoli: The phytoconstituent sulforaphane present in this vegetable inhibits the growth of breast cancer cells and prevents their initial growth. It is also present in cabbage, cauliflower and Brussels sprouts.

b. Sea vegetables: Seaweed contains high amount of naturally occurring iodine. It is important for proper thyroid function but it’s also shown potent effects in fighting the growth of breast cancer cells.

c. Brazil nuts high in selenium which is a powerful antioxidant and important for the prevention of the inflammation that accompanies breast and bone cancers.

d. Mushroom, yogurt, oat & barley, garlic, oat & barley contain β-glucan, a type of fibre with antimicrobial and antioxidant activities [38-40].

**References**


Abstract

Thyroid cancer is the most common of the endocrine malignancies and it represents <1% of all human malignant tumours. The annual incidence of thyroid cancer varies considerably by geographic area, age and sex. An escalating incidence during the last decades all over the globe has been reported [1]. This phenomenon is mainly due to an increase in micropapillary (<2 cm) histotype, while there is no substantial change in the incidence of the less common histological categories: follicular, medullary and anaplastic cancers. The increase is attributable to better detection of small papillary carcinomas as a result of improved diagnostic accuracy such as neck ultrasound, and US guided fine needle aspiration cytology FNAC [2]. The only established environmental risk factor for thyroid carcinoma is exposure to ionizing radiation, and the risk, particularly of papillary thyroid carcinoma, is greater in subjects of younger age at exposure time. An increased incidence of thyroid cancer in children and adolescents was observed in Ukraine, Belarus and certain regions of Russia as early as 4 years after the Chernobyl accident [3]. Despite increasing incidence, the mortality from thyroid cancer has tended to decline over the last three decades. It is unclear how much of the decline in mortality is due to better diagnosis rather than to improved treatment of thyroid neoplasm [4]. In order to plan an adequate surgical strategy, the diagnosis of differentiated thyroid carcinoma should be made preoperatively. In quite a few patients, however, the diagnosis will be made postoperatively. In these cases, the carcinoma is often still limited to the thyroid gland and the necessity to perform reoperation in the form of, completion thyroidectomy, remnantectomy, and/or lymph node dissection needs to be carefully assessed according to the definite histological evaluation of the surgical specimen [5].

Over the last decade, surgeons have witnessed dramatic changes in the surgical management of Differentiated Thyroid Carcinoma (DTC). This is not only a result of the introduction of new technologies in surgery but also a result of better understanding of the disease and its behaviour. DTC accounts for over 90% of all follicular-cell derived malignancies and is the commonest primary endocrine-related malignancy [6]. According
to the Hong Kong Cancer Registry, 2011 the age-adjusted incidence of DTC has doubled over the last 20-25 years with a similar trend being observed elsewhere [7,8]. Despite this, the cancer-specific mortality remains low with an overall 10-year survival above 90% [9]. However, recurrent or persistent disease after seemingly curative surgery poses a problem for both clinicians and patients [10]. Surgery shall remain to play pivotal role in the overall management of DTC. The primary aim of any new changes would be to further reduce and if possible, prevent the recurrences or the persistent diseases from occurring [11]. Such new changes include:

a. Adoption of new, innovative surgical approaches such as; endoscopic, robot-assisted and trans-oral thyroidectomy in surgical management of DTC in order to reduce the surgical morbidity, shorten hospital stay, and enhance patients’ satisfaction [12].

b. The use of several surgical adjuncts such as new alternate energy sources (Harmonic scalpel, Sonosurg, and LigaSureTM) Intraoperative Nerve Monitoring (IONM) and Rapid Intraoperative Parathyroid Hormone Assay (IOPTH) [13-17].

c. The routine adoption of prophylactic Central Neck Dissection (pCND) in DTC during total thyroidectomy [18].

These changes could lead to better patient outcomes when compared to the conventional open thyroidectomy with or without the help of the surgical adjuncts. Usually, thyroid cancer is a well treatable disease with a good prognosis. Current therapeutic protocols include surgery to remove the thyroid gland in part or totally, followed, if necessary, by application of I131. This radioisotope is accumulated in the thyroid remnant or even in the disseminated thyrocytes and destroys them by internal radiation. Another widely used therapeutic measure is the administration of Thyroxin (T4) to suppress the production of the Thyroid Stimulating Hormone (TSH) to reduce the proliferation rate of residual thyroid tissue or metastases. These therapeutic measures are so far the only ones with proven efficiency and prognostic relevance. They are successfully applied for the treatment of DTC [19]. However, in about 30% of the cases, dedifferentiation of the cancer is observed. Dedifferentiated tumours lose the TSH receptor (TSHr) and thus get insensitive to the growth-regulating effects of varying TSH levels; this obliterates any benefit from TSH suppression therapy by T4 [20].

**Diagnosis of DTC**

DTC is systematically diagnosed utilizing the triple assessment which comprises the clinical picture, investigations, and a tissue diagnosis using a Fine Needle Aspiration Cytology “FNAC”. Triple assessment usually gives a diagnosis in the majority of cases [21]. History and physical examination usually reveal a painless neck swelling that moves with swallowing. A past history of exposure to ionizing radiation and a family history of thyroid cancer need to be documented as well as a previous history of neck surgery. Thyroid ultrasound is a widely used technique as a first-line diagnostic procedure for detecting and characterizing nodular thyroid disease. US features associated with malignancy are: hypo-echogenicity, micro-calcifications, absence of peripheral halo, irregular borders, solid aspect, intra-nodular blood flow and shape more taller than wide [22]. Though operator dependant US guided FNAC is a useful test for the diagnosis of thyroid nodules. Being operator dependant its sensitivity and specificity vary from 56% to 90%. It has decreased the number of unnecessary surgical procedures on benign thyroid lesions and increased the prevalence of thyroid cancer in various series [23]. The FNAC results are used to recommend non-operative and operative management of the tumour. The pathologist, however, often cannot distinguish reliably between benign and malignant thyroid lesions
on the basis of cytology alone. Therefore, the only way to make an accurate distinction between benign and malignant disease is based on final permanent section histopathology examination of the thyroid nodule and the surrounding tissue [24]. This 2-stage approach may result in an inadequate initial surgery necessitating reoperations with a greater morbidity than more extended primary surgical procedures [25]. In the absence of a clear cytological diagnosis, there is a diagnostic “gray zone” in FNAC resulting in cytological Indeterminate Thyroid Nodules (ITN). By definition, indeterminate cytology integrates smears not convincingly benign and not diagnostic of a neoplastic or a malignant process namely (follicular lesion/atypical of undetermined significance, follicular neoplasm/suspicious for follicular neoplasm; or suspicious for malignancy). In 5-75% of patients, indeterminate cytology is associated with thyroid malignancy, including Follicular Thyroid Carcinoma (FTC), “pure” Papillary Thyroid Carcinoma (PTC), or the follicular variant of PTC. The aforementioned features of indeterminate cytology demonstrate both the challenge and importance of differentiation between benign and malignant lesions in applying additional clinical parameters [26]. Debate persists in the literature about the correlation of certain clinical patterns with the risk of malignancy. Although several studies suggest that sex, age, and tumour size could be predictive of malignancy in ITN this finding has not been confirmed by others [27]. Furthermore, there is still controversy regarding the accuracy of intraoperative Frozen Sections (FS) in determining the extent of initial operation in patients with ITN [28]. The limitations of FNAC include inadequate samples and follicular neoplasia. In the event of inadequate samples, FNAC should be repeated, while in thecae of follicular neoplasia, with normal Thyroid Stimulating Hormone (TSH) and ‘cold’ appearance at thyroid scan, surgery should be considered. Most of these nodules are scintigraphically cold, and most cold thyroid nodules are benign. Yet a scintigraphically normal or hot nodule does not exclude the presence of a DTC [29]. In papillary thyroid cancer, the initial symptom may be enlarged cervical lymph nodes as a sign of metastatic involvement, which used to be mistakenly called in the past “lateral aberrant thyroid”. An FNAC should be performed if thyroid nodules are clinically suspicious to be malignant (e.g. solid, rapidly growing) [30]. FNAC might also be helpful in the evaluation of suspicious lymph nodes. Cytological features of papillary thyroid carcinoma are diagnostic by FNAC [31]. In follicular thyroid carcinoma, however, the contribution of FNAC in making the diagnosis is limited because vascular and capsular invasion need to be seen before such a diagnosis is established [32]. At primary operation, extensive imaging techniques are often not required unless invasion of the trachea or oesophagus or distant metastases are suspected. Ultrasound, however, should be performed to identify the extent and localization of the primary and coexisting thyroid nodules and to diagnose enlarged cervical lymph nodes. Radioiodine cannot be used as a diagnostic tool unless the thyroid gland has been removed. Prior to extensive operation, distant macrometastasis, most often found in lung and bones, should be ruled out. In recurrent disease, other imaging techniques have been shown to be helpful (e.g. Technetium-99m, Thallium-201, FDG-PET) [19]. Recently, it has been reported that by molecular testing for thyroid nodules (BRAF, RAS, RET/PTC and PAX8/PPARY mutations), the presence of any mutation was a strong indicator of cancer because 97% of mutation-positive nodules had malignant diagnosis at histology. BRAF on the other hand, thyroid function test and Thyroglobulin (Tg) measurement are of little help in the diagnosis of thyroid cancer [33]. In a recent study in Saudi Arabia a group of researchers (Hans-Juergen Schulten et al) looked at BRAF mutation in thyroid tumours from an ethnically diverse group, they identified BRAF mutation from one of 69 Follicular adenoma, 72 of 115 (63%) PTC, seven of 42 (17%) FVPTC, 10 of 56 (18%) micro PTC, one of 17 (6%) FTC and FTC harboured a k061E mutation. They concluded that older age is manifold associated with unfavourable tumour markers. The K601E identified in a PTC, FVPTC, and FTC seems to be more distributed among different histological types of thyroid cancer than previously thought [34].
Staging and Surgical Strategies for the Management of DTC

The AJCC classification of DTC is based on the TNM system, which assesses three components: the size and extent of the primary tumour (T), the presence or absence of regional lymph node metastatic involvement (N), and the presence or absence of distant metastatic lesions (M). These three categories are further subdivided numerically; thus, progressive increase in tumour size and involvement can be indicated. The TNM classification may be either clinical (cTNM), based on evidence including FNA biopsy acquired before treatment, or pathologic (pTNM), based on available intraoperative and surgical pathologic data. The postoperative TNM classification is preferable because the tumour can be categorized histologically and can be precisely measured, and the extra thyroid invasion can be unequivocally demonstrated [35].

Surgery is the treatment of choice in DTC. The extent of surgery in regard to thyroid gland and lymph nodes, however, varies from conservative treatment to radical approaches. In papillary thyroid carcinoma, some surgeons advocate routine total thyroidectomy as the treatment of choice for the following reasons: [19,36]

a. Papillary thyroid carcinoma is often multifocal (>25%).
b. Small lesions may grow aggressively with the potential of dedifferentiation.
c. Rate of local recurrences is increased after less than total thyroidectomy.
d. An experienced surgeon can perform a total thyroidectomy with minimal or no long-term complications.
e. Completion thyroidectomy of thyroid remnants (remnantectomy) may be associated with a higher morbidity.
f. Measurement of thyroglobulin can be used during follow-up.
g. Radiiodine can be used for diagnostic and therapeutic purposes of metastatic disease.
h. Ablation of gross thyroid remnants with radiiodine can be associated with pain.

Those who advocate less than total thyroidectomy their recommendations depend on the following reasons: [37,38]

a. Scoring systems enable to identify low risk patients who have a 20-year survival rate of 99% and a 20 year disease-free survival of >90%.
b. Low risk (<1%) of conversion of differentiated thyroid carcinoma to undifferentiated (anaplastic) thyroid carcinoma.
c. No difference in survival as compared to total thyroidectomy.
d. Local recurrences can be managed by reoperation.
e. Development of a recurrent thyroid cancer in the remnant thyroid lobe is considerably less common than the reported incidence of microscopic disease.
f. Decreased morbidity after lobar or subtotal thyroidectomy as compared to total thyroidectomy.
g. If necessary, ablation of the thyroid remnant (of less than one cm) with radiiodine can be accomplished with no morbidity.

The initial treatment of DTC diagnosed preoperatively is usually total thyroidectomy. Less extensive surgical procedures e.g. lobectomy may be accepted in cases of unifocal DTC.
diagnosed at final histology after surgery performed for benign thyroid disorders, provided that the tumour is small intrathyroidal and of a favourable histological type (classical papillary or follicular variant of papillary or minimally invasive follicular). In the case of widely invasive follicular cancer at final histology, completion thyroidectomy is indicated [39].

In our institution we manage our patients by adopting consensus-based-guidelines for the treatment of patients with DTC. The cases are discussed during a weekly Multi-Disciplinary Team (MDT). The MDT strictly follows up to date evidence-based international guidelines that include:

a. Initial adequate surgery,

b. optimization of TSH suppression,

c. Radioactive iodine therapy and excision of residual thyroid tissue of more than 2 cm size,

d. Dissection of ipsilateral FNAC positive lymph nodes and those lymph nodes which are reported suspicious on ultrasound scan.

e. We reset selected metastases and we use bisphosphonates for bone metastases.

When it comes to radio iodine ablation therapy we use a staging system according to which patients are classified from 1 to 4:

Stage 1: Primary tumour smaller than 1.5 cm in diameter

Stage 2: Primary tumour 1.5 - 4.4 cm or presence of cervical lymph node metastases, or more than three intra-thyroidal foci of tumour

Stage 3: Primary tumour at least 4.5 cm, or presence of extra thyroidal invasion

Stage 4: Distant metastases

Patients who have stage one and two may not need radioactive ablation. Although most patients do well, there is still considerable contention related to the extent of thyroidectomy and postoperative management of these patients. In the absence of randomized controlled trials in this area of controversy and that the results of the ongoing ones will take so long to give out results, and given the typically prolonged course and the relative infrequency of these tumours, therefore the only way to resolve this issue is by discussing the grey area cases each at its own merits in a broad based Multi-Disciplinary Teams armoured by international and local guidelines, since most of the information about the treatment comes from studies of large patients’ cohorts in which therapy has not been randomly assigned. This accounts for much of the disagreement about managing differentiated thyroid cancer. Total thyroidectomy is the standard of care in our institution for DTC except for unifocal non-invasive micro papillary carcinoma (less than 1 cm), when a lobectomy with an isthmusectomy would be enough without radioactive iodine ablation [22].

**Surgical Strategies of Cervical Lymph Nodes Management**

Metastatic disease to regional lymph nodes is common in patients with papillary thyroid cancer, particularly in autopsy series, where rates can exceed 90 percent. Palpable nodal disease is present in approximately 5 to 10 percent of patients with papillary thyroid cancer; a preoperative neck ultrasound can detect lymph node disease in up to 30 percent of patients [40-43]. Standard histological staining techniques typically reveal positive lymph nodes in 20 to 50 percent of patients undergoing an elective node dissection for papillary
thyroid cancer [19]. However, after immunohistochemical evaluation i.e. cytokeratin stain, up to 90 percent of patients will have microscopic metastatic disease [44-46]. It appears that many patients have microscopic regional lymph node disease that never becomes clinically apparent. This regional Lymph Node Metastases (LNM) have been demonstrated in as many as 80% of patients with PTC, but only about 35% of these patients have clinically evident nodal metastases at the time of their initial surgical procedure. In contrast, less than 5 percent of patients with follicular thyroid cancer develop nodal metastatic disease; the haematogenous rather than the lymphatic route are the primary pathway for metastasis [47]. While these metastatic nodes do not necessarily portend a worse prognosis at presentation, excision can minimize lymph node metastases, recurrence and reoperation. There is widespread consensus by specialty societies and recognized international experts in thyroid carcinoma that surgery is the best treatment of cervical lymph node metastases [48]. The current American Thyroid Association guidelines for the management of PTC states in (Recommendation No. 21) that “preoperative neck ultrasound of the contra lateral lobe cervical (central and bilateral) lymph nodes is recommended for all patients undergoing thyroidectomy of malignant cytological findings on biopsy.” Moreover regarding lateral neck compartment lymph node, the guidelines state in (Recommendation No.27) “for those patients in whom nodal disease is evident clinically, on preoperative ultrasound, or at the time of surgery, surgical resection may reduce the risk of recurrence and possibly mortality. In (Recommendation No. 28) “Lateral neck compartment lymph node dissection should be performed for patients with biopsy proven metastatic cervical lymphadenopathy detected clinically or by imaging.” [19].

Both the American Association of Clinical Endocrinologists and the American Association of Endocrine Surgeon agree that it is appropriate to remove all the enlarged lymph nodes on both the central and the lateral neck compartments [49]. Central neck lymph node dissection plays an important role in the appropriate treatment of papillary thyroid cancer at initial presentation and in cases of recurrent disease. Surgeons caring for this group of patients should have familiarity and skill with this procedure. Papillary thyroid cancer and the follicular variant of papillary thyroid cancer have a propensity for cervical lymphatic spread that occurs in 20% to 50% of patients on standard review of surgical pathologic specimens and in 90% of those examined for micro metastases. The spread of tumour cells occur in a predictable pattern that initiates in the perithyroidal lymph nodes of the central neck and progresses to the lymph nodes of the lateral cervical compartments and the superior mediastinum [50]. “Skip” metastases to the lateral compartment without central neck nodal involvement is rare but do occur. Patients with nodal metastasis have higher rates of persistent and recurrent disease during postoperative surveillance. The impact of nodal metastasis on overall survival remains debatable; several studies have demonstrated no difference in mortality, while two large population-based studies have shown increased mortality in patients with regional lymph node metastasis [51].

The established primary treatment of papillary thyroid cancer according to the ATA guidelines is total thyroidectomy for all tumours larger than 1 cm, while thyroid lobectomy sufficient for tumours smaller than 1 cm. The ATA consensus statement also recommends therapeutic central neck dissection in patients with clinically involved nodes and prophylactic central neck dissection in advanced primary tumours (T3 or T4) without evidence of nodal involvement. Radioactive iodine ablation plays an important role in adjuvant treatment following thyroidectomy for some subgroups of patients based on risk of recurrence. This treatment algorithm achieves extremely low death rates; however, the rates for cervical lymph node metastasis and recurrence remain significant [19]. Despite the ATA recommendations, there is still controversy regarding the ideal surgical management of the central neck lymph nodes in patients with papillary thyroid cancer.
Preoperative ultrasound is valuable to detect and localize precisely non palpable lateral neck lymph nodes in 15% of patients. Further; even when nodes are palpable, the ultrasound alters the extent of resections in 40% of patients by its ability to evaluate the entire neck. Approximately 40% to 45% of patients who have a node dissection in the central compartment prove to have positive nodes. The ultrasound is rather insensitive for central nodes prior to the first time cervical exploration [52,53]. The benefit of prophylactic central node dissection in the absence of evidence of nodal disease is controversial. There is no evidence that it improves recurrence or mortality rate, but it permits an accurate staging of the disease that may guide subsequent treatment and follow-up. However, it is not indicated in follicular thyroid cancer; compartment oriented micro-dissection of lymph nodes should be performed in cases of preoperatively suspected and/or intraoperatively proven lymph node metastases in follicular thyroid cancer [54].

**Surgical Techniques for Cervical Nodal Dissection**

The surgical technique for cervical nodal dissection for papillary thyroid cancer should include a systematic or en bloc nodal basin dissection rather than a selective or “berry picking” dissection due to higher rates of persistent and recurrent disease with the later approach [55]. The general approach to the dissection of lymph nodes in PTC is:

a. First the submental, submandibular, parotid and retro auricular nodes are virtually never dissected in PTC.

b. Second, the central compartment V1is dissected routinely and completely in PTC.

c. Third, the key lateral compartment is III, IV and the anterior aspect of level V posterior to the sternocleidomastoid muscle, but not formally extending the dissection to the trapezius muscle. These are the lateral compartments that are routinely dissected en bloc in PTC for LNM. If, however, by either palpation or preoperative ultrasound, positive lymph nodes are suspected on level II, this compartment is included in the en bloc dissection.

d. Fourth the mediastinal nodes below the innominate artery are rarely dissected in PTC [56].

The ATA consensus statement regarding the terminology and classification of the central neck defines the central compartment nodal dissection as all perithyroidal and paratracheal soft tissue and lymph nodes with borders extending superiorly to the hyoid bone, inferiorly to the innominate artery, and laterally to the carotid arteries [19]. The inclusion of level VII nodes in the superior mediastinum with the central neck dissection should be noted, as this is often a site of persistent disease following central neck dissection. Moo et al [57] compared ipsilateral vs bilateral central neck dissection for papillary thyroid cancer and concluded that an ipsilateral dissection was sufficient in tumors less than 1 cm, while tumors larger than 1 cm required bilateral central neck dissection based on the high incidence of contralateral central neck disease in a retrospective analysis of the pattern of nodal metastases in surgical specimens. Some additional studies demonstrated that ipsilateral central neck dissection was adequate for tumors larger than 1 cm [58]. If lateral cervical metastases are present in levels II through V, a bilateral central nodal dissection should be included with the modified radical neck dissection to remove the presumed central neck nodal disease based on described patterns of nodal spread [59]. Prophylactic or routine central neck dissection for patients with papillary thyroid carcinoma is defined as complete excision of the level VI and VII lymph nodes in patients with no evidence of nodal involvement after pre-operative clinical and imaging evaluation. The role of prophylactic central neck dissection remains a contentious issue regarding its benefits and risks, and several reports have reviewed this subject [60, 61]. Several single institution retrospective
cohort studies on total thyroidectomy alone vs with prophylactic neck dissection, as well as a meta-analysis of these studies, have reported mixed results [58,63-66]. Proponents of prophylactic central neck dissection at the time of initial thyroidectomy cite the high incidence of cervical lymph node metastasis and the associated increase in recurrence rates with the possibility of decreased survival. The low sensitivity of preoperative ultrasound evaluation and intraoperative assessment to accurately detect lymph node involvement is also used as a rationale for routine central neck dissection [67-69]. The addition of central neck dissection to initial total thyroidectomy can provide valuable staging information and has been shown to upstage approximately a third of patients older than 45 years of age to stage III disease in two retrospective reviews [70,71]. This upstaging has important implications for further treatment as those with nodal metastasis are likely to receive higher doses of I131 ablation treatment, while those with small noninvasive tumors without nodal disease can forgo I131 ablation. The evidence to support prophylactic dissection due to decreased recurrence rates and improved survival is sparse and is primarily composed of a prospective population-based study from Sweden [72]. This study demonstrated that the rate of death due to thyroid cancer, which ranged from 8.4% to 11.1%, was reduced to 1.6% in patients who underwent central neck dissection compared to contemporary controls. However, several retrospective cohort studies have shown no difference or only a slight improvement in recurrence or survival rates.

The complications of central neck dissection include injury to the recurrent laryngeal nerve or the external branch of the superior laryngeal nerve, which occurs in 1% to 2% of patients based on several studies. Small retrospective studies have shown that the addition of central compartment lymphadenectomy to total thyroidectomy for thyroid cancer has not increased nerve injury rates in experienced hands [73]. In cases of reoperative central lymph node dissection after either previous thyroidectomy or central node dissection, reports have noted increased nerve injury rates ranging from 1% to 12% [74]. Temporary hypoparathyroidism following central neck dissection occurs in 14% to 40% of cases depending on the definition of hypoparathyroidism used in the study [75]. The higher incidence of temporary hypoparathyroidism is likely due to the increased incidence of parathyroid re-implantation and inadvertent inclusion of parathyroid glands in the nodal dissection. Reports are mixed regarding the risk of permanent hypoparathyroidism. A meta-analysis of retrospective studies reported a 1.2% incidence as defined by the requirement for calcium supplements greater than 6 to 12 months postoperatively; however, none showed a statistically significant difference in total thyroidectomy with or without central neck dissection [66].

Reoperative central neck dissection is defined as removal of all remaining soft tissue in the level VI and VII compartments in a patient who has undergone previous thyroidectomy or central lymph node dissection. This is often indicated for patients with papillary thyroid cancer who are noted to have central neck lymph node involvement on surveillance examination or imaging studies after completion of radioactive iodine ablation therapy. The goal of re-operative neck dissection is the removal of all persistent or recurrent cervical disease. It is important to closely evaluate patients for the presence of additional pathologic lymphadenopathy in the lateral neck and superior mediastinum. Combining imaging modalities with cervical ultrasound and cross-sectional imaging with CT or PET/CT can help to guide operative planning and to determine the necessary extent of nodal dissection. Ultrasound and physical examination will often miss pathologic lymphadenopathy the superior mediastinum, which can be detected with cross-sectional imaging and can usually be removed via a cervical incision with caudal extension of the central compartment lymphadenectomy. Preoperative laryngoscopy should be performed before all re-operative
procedures to determine the presence of recurrent laryngeal nerve injury, which can affect the approach to reoperative nodal dissection.

Given the challenging nature of reoperative neck dissection, consideration of recurrent laryngeal nerve monitoring and special care to preserve the parathyroid vascular pedicles originating from the inferior thyroidal arteries are important.

**Patients Stratification and Risk Assessment of DTC**

The most important risk factors for DTC recurrence and cause specific mortality are age at time of initial assessment, tumour size, and presence of extra thyroid invasion, and presence of distant metastatic lesions. lymph node metastatic lesions at the time of initial examination do not increase the risk of death but do increase the risk of local and regional recurrences. Although nodal metastatic lesions are uncommon in FTC, their presence may indicate a worse prognosis [76]. Minimally invasive FTC characterized by capsular invasion alone rarely spreads or causes death. The prognosis is slightly worse when, at most, a few blood vessels are invaded. FTC with extensive vascular invasion denotes much worse prognosis [77]. Several staging systems have been developed by authoritative centres. Each of these staging systems provides good risk stratification based on data available shortly after initial therapy. The ATA first risk stratification category is low risk, when there is no local or distance metastases, all the macroscopic tumour has been resected, no tumour invasion of loco-regional tissue or structures, no aggressive histology or vascular invasion, and if radioactive iodine is given, there will be no uptake outside the thyroid bed in a post therapeutic Whole Body Scan (WBS). The intermediate risk category is when microscopic invasion of tumour into the parathyroid soft tissue at initial surgery, cervical LN metastases or radioactive I131 outside the thyroid bed on the post therapeutic WBS, or tumour aggressive histology or vascular invasion. And a patient is labelled high-risk category when there is macroscopic tumour invasion, or incomplete tumour resection, or distant metastases or when thyroglobulin out of proportion to what is seen on the post ablative scan [19]. The European Thyroid Association on the other hand categorizes patients as low risk when the surgery is complete, or the patient has unifocal microcarcinoma of less than one cm with no extension beyond the thyroid capsule and without lymph node metastases. Their intermediate risk patients are those with no local or distant metastases, no tumour invasion of loco-regional tissues or structures and with no aggressive histology or vascular invasion. And the high risk patients are those who had less than total thyroidectomy, the tumour is invading loco-regional tissues or structures, the cervical lymph nodes harbour metastases, or the presence of distant metastases or aggressive histology or vascular invasion [76]. This risk stratification is utilized to facilitate the use of radioactive iodine post-surgery using its activity in ablating any remnant thyroid tissue and potential microscopic residual tumour. This procedure decreases the risk of loco-regional recurrence and facilitates the long surveillance based on serum Tg measurement and diagnostic radioiodine WBS. In addition, the high activity of I131 makes it possible to obtain a highly sensitive post-therapeutic WBS. So according to several guidelines the recommendations for small (less than one cm) remnant thyroid ablation are modulated on the basis of risk factors. Radioactive iodine ablation is indicated in high risk patients, whereas it is not indicated in low risk patients. In patients at intermediate risk radioactive iodine remnant ablation may be indicated, but the decision must be individualized. The surgical strategy for large size thyroid remnant (>1-2 cm) in high or intermediate risk patients; thyroid tissue remnant excision (remnantectomy) may be indicated prior to radioactive iodine ablation of the thyroid remnant.

Surgery is the treatment of choice if lymph node metastases are present. Lymph node metastases of papillary thyroid carcinoma are often macrometastases; however, they can be
very small in size. Diagnosis might be only possible histologically. Lymph node dissection should therefore not only include obviously enlarged lymph nodes but also the whole adipose and connective tissue in order to dissect also the very small, possibly metastatic lymph nodes within this compartment [78]. Though the primary therapy for differentiated thyroid cancer is surgery, yet considerable controversy exists about how much thyroid tissue should be removed at the initial operation, and there are no prospective randomized clinical trials to provide guidance for selection of the optimal operation. However, an increasing body of evidence suggests that most patients with papillary and follicular thyroid cancer are best treated by total thyroidectomy [79]. Even though surgery is accepted as the treatment of choice in DTC no consensus exists neither regarding the extent of thyroidectomy nor regarding the extent of lymph node dissection [39]. In contrast, the prognostic significance of lymph nodes remains controversial. While it has been repeatedly shown that their initial presence is correlated with tumor recurrence, most studies could not prove a significant influence on survival [80].

Recurrence can be identified based on clinical findings, biochemical activity, or radiological studies. Locations for recurrence can be divided into central recurrence, in the thyroid bed or central compartment lymph nodes; lateral neck recurrence in lymph nodes or soft tissues and distant metastases [81]. The significance of nodal recurrence on morbidity and survival is presently unclear, particularly for low-volume disease. Due to the favorable prognosis of DTC, traditional means of staging cancer based on overall survival have now been replaced in many institutions with stratification based on risk of recurrence, as seen in the most recent ATA guidelines [19]. The aim of the management of DTC is focused on the control of current disease while minimizing morbidity to the patient. Current literature quotes recurrence rates of DTC up to 30%, the majority of which will occur within 10 years of primary surgery. Recurrence has been kept stable due to improved pre-operative screening and more well defined surgical techniques for dealing with primary surgical management of DTC. Factors leading to a higher likelihood of recurrence include increasing age, male gender, increasing tumour size, extra-thyroidal extension, and nodal disease at time of primary surgery and aggressive histological type. Most of this data relates to PTC, which is by far the more common of the DTC. Studies specifically looking at FTC are less frequent in the literature. When a patient presents with recurrent DTC it can be a time of significant anxiety for both the patient and the treating physician. A balance must be achieved between providing adequate information and reassurance to the patient, while reducing morbidity and mortality. Time spent with the patient and their family will help them to understand the rationale for the different treatment options and help them to feel that they are a part of the decision-making process. Once the recurrence has been well established, clinically, by imaging or by a rising Tg and anti Tg levels. Once the recurrence is established then the traditional treatment options for recurrent DTC includes [81].

1. Observation
2. Radioactive Iodine I131 Ablation (RAI)
3. Surgical resection of the involved regions
4. External Beam Radiation Therapy (EBRT)

Technological Innovations Serving the Surgical Strategies of DTC

New technologies have had an important influence in the management of DTC. In addition to improving the preoperative diagnostic accuracy and cancer staging with various imaging modalities, the techniques of thyroid cancer surgery have been refined and evolved in this
era of technological advancement. In applying these new technologies, it is believed that surgical morbidity can be further reduced, hospital stay shortened, and patient satisfaction enhanced [82]. The developments of the past several years have enabled thyroid surgeons to achieve new boundaries in patient care. Although many of the new technologies have yet to prove itself and stand the test of time resoundingly to reduce surgical complications. Their applications have enabled surgery with minimal dissection and smaller incisions (in the case of endoscopic and robotic surgical techniques), [12] and reduced operating time (in the case of devices for improved hemostasis). The reliability of PTH monitoring, as well as robotic and endoscopic techniques of dissection has also facilitated shortened hospitalization and the feasibility of same day surgery [83]. These innovations include the development of various endoscopic thyroidectomy techniques, the addition of the Da Vinci robot surgical system [84-86], as well as the use of operative adjuncts such as Intraoperative Neuromonitoring (IONM) and quick Intraoperative Parathyroid Hormone (IOPTH) [87].

The application of endoscopic visualization of the thyroid gland has allowed surgeons to perform safe surgery from extra-cervical skin incisions [82]. Although short-term outcome studies in various endoscopic techniques demonstrated comparative results as conventional open thyroidectomy for DTC this particular operative approach is indicated for selected patients only and the benefit is still considered marginal with concerns of higher associated cost and longer operating time in performing these procedures. Although the robot procedures might offer some theoretical advantages over the endoscopic procedures, better-designed prospective comparative studies are required. Despite the lack of strong evidence for the benefit of routine use of IONM, there is a trend toward improved RLN protection and reduced iatrogenic RLN injury. As a surgical adjunct, IOPTH is being actively sought as a cost-effective tool for predicting postoperative hypocalcaemia in patients undergoing total thyroidectomy for thyroid cancer [88].

**Conclusion**

Considering all the controversy and debate, Differentiated Thyroid Cancer remains prognostically favourable in comparison to other forms of malignancy, with disease-specific death rates as low as 1.3%. It also remains common knowledge that DTC is a rare disease with a good to excellent prognosis, dedifferentiation is quite known to happen though at a low incidence, recurrence of the disease is partly iatrogenic and partly due to the disease biology. The surgical and the endocrine community are still far away from finding the ideal curative treatment for DTC. Though a lot of innovation are currently available and is in interplay, yet a lot is expected to be seen in the near future from the biologist and the molecular biologist about the way forward in the non-surgical modalities for the treatment of DTC.

The environmental factors known to cause this disease were seen many times but once at a wider scale in Chernobyl and Belarus, sending a very strong message about human safety and a clean green environments for the sake of our future generation.

The last but by no means least is to emphasize the importance of managing DTC in well-designed multidisciplinary teams fully authorized to take decisions on individual patients and on the institutions' policies on how to treat patients with DTC; of course armored by up to date evidence based knowledge inline and online with the most recent and up to date international and local guidelines.

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Abstract

The parathyroid glands require an expert knowledge from the surgeon’s side to identify the unusual anatomic locations of the enlarged and sometimes the normal parathyroid glands. This is crucial to the operative success during both initial and reoperative parathyroid surgical exploration. The wide range of parathyroid anatomic variations may make it difficult to predict a patient’s anatomy preoperatively. The parathyroid glands were first identified by Sir Richard Owen in the Great Indian Rhinoceros in 1850 [1]. They were identified in humans by Ivar Sandstrom, a Swedish medical student, in 1880. The first parathyroidectomy was reported by Felix Mandl in 1929 [2], 30 years prior to the isolation of human parathyroid hormone. The success of the surgical management of parathyroid disease is based on a high index of suspicion, a sound clinical diagnosis, an accurate biochemical diagnosis and the surgeon’s understanding of the significant embryologic variations in parathyroid anatomy.

Embryology & Surgical Anatomy

The parathyroid glands arise from endodermal epithelial cells, in conjunction with the thymus. The superior parathyroid glands are derived from the fourth branchial pouch. These glands are closely associated with the lateral lobes of the thyroid and have a short line of embryologic descent [3]. The inferior parathyroid glands are derived from the third branchial pouch. These glands are closely associated with the thymus and have a longer line of embryologic descent, which leads to more variability in their anatomic position. The inferior parathyroid glands can be found as high in the neck as the carotid sheath and can also be found in the anterior mediastinum or even the pericardium. However, the majority of inferior parathyroid glands are found near the inferior pole of the thyroid [4]. The locations of ectopic parathyroid glands are related to the common origins of parathyroid, thyroid, and thymic tissue. The third branchial pouch contributes to thymus development as well as parathyroid and thyroid development. Both the third and fourth branchial pouches also contribute to thyroid development. Normal size of the parathyroid glands is approximately 5 by 4 by 2 millimeters in size and weighs 35 to 50 milligrams. Enlarged parathyroid glands can be 50 milligrams to 20 grams in weight, most typically weighing about 1 gram and 1 centimeter in size [3].
The macroscopic appearance of parathyroid glands can vary considerably. The color varies from light yellow to reddish-brown tan colour. Parathyroid glands are oval, bean shaped, elongated or spherical. Occasionally the glands are bi-lobed or multilobed. Most (84 percent) patients have four parathyroid glands, two superior and two inferior glands. Additional glands are found in 13 percent of patients and only three glands in a very small number of patients (≤3 percent). The terms “superior” and “inferior” refer to a gland’s embryologic origin, rather than the gland’s location in the neck [5].

The parathyroid glands are usually in close association with the thyroid gland. Although there is significant variability in the position of the glands, they are usually symmetrical. The superior glands are symmetrical in 80 percent of cases and inferior glands are symmetrical in 70 percent of the times. Normal superior parathyroid glands are usually located on the postero-lateral surface of the middle to superior thyroid lobe. They lie under the thyroid superficial fascia, posterior to the recurrent laryngeal nerve and can be visualized by carefully dissecting the thyroid capsule in this region. These glands also can reside inside the thyroid capsule, just superior and medial to the posterior tubercle of Zuckerkandl of the thyroid lobe [6].

The recurrent laryngeal nerve is always anterior to the superior parathyroid gland. The superior parathyroid glands are usually 1 to 2 centimeters superior to the junction of the recurrent laryngeal nerve with the inferior thyroid artery and within one centimeter of the entry point for the recurrent laryngeal nerve into the ligament of Berry and the cricoid cartilage.

Superior parathyroid glands can be undescended, or can be parapharyngeal, retropharyngeal, or retrotracheal within the middle cervical/mediastinal compartment. Enlarged parathyroid glands can travel straight down the tracheoesophageal groove or the retropharyngeal space into the chest. The two inferior parathyroid glands reside in the anterior mediastinal compartment, anterior to the recurrent laryngeal nerve. They are most often found in the thyrothymic tract, or just inside the thyroid capsule on the inferior portion of the thyroid lobes [7].

An ectopic parathyroid gland that fails to have full migration during normal development is termed “undescended.” The ectopic gland may be one of the four parathyroid glands or it may be a supernumerary gland. An ectopic superior parathyroid gland may be undescended and located at the piriform sinus. Superior parathyroid glands can also be intrathyroidal, but less commonly than inferior parathyroid glands. Ectopic inferior parathyroid glands are most often found in the thymus or mediastinum. An undescended inferior parathyroid gland may be located anywhere within the carotid sheath. They can also be located intrathyroidal.

The majority of supernumerary glands are small, rudimentary, or divided. However, when enlarged, these additional glands may be responsible for persistent hyperparathyroidism after failed parathyroid exploration, especially in patients with secondary hyperparathyroidism or hyperparathyroidism associated with familial syndromes. Supernumerary glands can reside anywhere from behind the thyroid down to and including within the thymus, representing the line of descent of thymic tissue during embryologic development. The most common location is within the thymus or in relation to the thyrothymic ligament. The remaining supernumerary glands are usually found in the vicinity of the mid-thyroid lobe between two other glands [5]. In most patients, the inferior and superior parathyroid glands will both be supplied by branches of the inferior thyroid artery. Each parathyroid gland usually has its own end-artery. Most parathyroid glands have a single arterial supply. The venous drainage of the parathyroid glands consists of the superior, middle, and inferior thyroid veins that drain into the internal jugular vein or the innominate vein. During thyroid surgery, the
surgeon should try to preserve all of the parathyroid glands in situ with adequate blood supply whenever possible. However, the blood supply may not be adequate following dissection of the thyroid gland, and the parathyroids are not always clearly identified. It can be difficult to make a reliable intraoperative determination of individual parathyroid function and patients may experience transient hypoparathyroidism despite having all four parathyroid glands preserved. Ligation of the branches of the inferior thyroid artery close to the thyroid parenchyma and medial to the recurrent laryngeal nerve may help preserve intact parathyroid vascularity.

**Diagnosis and Management of Parathyroid Problems**

Before offering parathyroid surgery, the surgeon must carefully review and confirm the preoperative diagnosis to avoid unnecessary surgery. Errors in diagnosis are a major cause of failed initial exploration. Initial evaluation includes a detailed history and a thorough physical examination. The history shall include personal and family history of other endocrinopathies that may suggest a MEN syndrome or isolated familial hyperparathyroidism. Systems review and review of the biochemical diagnosis are mandatory and the images requested should be reviewed with a radiologist before contemplating surgery.

Hyperparathyroidism occurs as a result of a parathyroid adenoma in 80-90% of cases, parathyroid hyperplasia in 10-20% and rarely due to parathyroid carcinoma. In the hands of experienced surgeons, cervical exploration and excision of the hyperfunctioning parathyroid gland(s) provides symptomatic cure as well as biochemical normalization in greater than 95% of patients with primary hyperparathyroidism [8]. Optimal preoperative localization has made minimal invasive parathyroid surgery like unilateral neck exploration, video assisted or videoscopic parathyroidectomy possible. These are being increasingly performed and produce equal cure rates with lesser morbidity and complications, provided preoperative localization of the abnormal parathyroid glands was accurate [9]. High resolution ultrasonography, a quick, convenient and inexpensive imaging modality, and Tc-99m Sestamibi Scintigraphy are the most common imaging modalities used for preoperative localization of abnormal parathyroid glands. Studies have shown that high resolution ultrasound has a sensitivity ranging from 77 to 80% in diagnosing a single adenoma [10-14].

**Localization of the Diseased Gland & the Choice of Surgical Procedure**

There is considerable variability in the location of the parathyroid glands. Thus, identification of all glands often requires careful exploration of the upper mediastinum, thyroid gland, carotid sheaths, and retroesophageal area. If four glands are not identified, persistent or recurrent hyperparathyroidism occurs in as many as 14 percent of cases. Because of these difficulties, radionuclide scanning may help detect hyperplastic parathyroid glands. Technetium-sestamibi scanning has been extensively studied in patients with primary hyperparathyroidism [13]. On the contrary, little is known concerning its accuracy in patients with secondary hyperparathyroidism due to renal failure. It remains to be determined whether or not the preoperative use of such localization techniques in patients with hyperparathyroidism due to renal failure affects outcomes, such as the rate of surgical complications and long-term failures [15].

Once the decision to perform a parathyroid operation is made, the surgeon must decide whether a standard bilateral neck exploration or a more focused minimally invasive parathyroidectomy is appropriate [16]. The ultimate goal of parathyroid surgery is to attain a durable biochemical cure, defined as eucalcemia at six months postoperatively.
The standard surgical approach has traditionally been bilateral neck exploration under general anesthesia. However, with increased experience and availability, minimally invasive parathyroidectomy techniques combined with intraoperative PTH monitoring have emerged and appear to be as effective as bilateral cervical exploration in appropriately selected patients [17, 18].

Bilateral parathyroid exploration has stood the test of time to exclude or identify multiglandular disease with high surgical cure rates between 95 to 99 percent. Therefore bilateral neck exploration is indicated in the following situations:

- For patients with negative (non-localizing) preoperative imaging studies.
- When bilateral foci are detected.
- Most forms of hereditary hyperparathyroidism due to the predictable involvement of multiple glands.
- For young male patients with apparent sporadic primary hyperparathyroidism because they are at increased risk for MEN-1.
- Concomitant thyroid disease requiring surgical resection, such as biopsy proven papillary thyroid cancer.
- In pregnant patients due to the radiation required for some localization studies. However, if ultrasound expertise is available and the results show an apparent single adenoma, a focused approach can be planned.

Focused parathyroid exploration when combined with the use of intraoperative PTH monitoring, minimally invasive parathyroidectomy techniques result in excellent outcomes that are comparable to a traditional bilateral cervical exploration [19]. Localization results inform the surgeon where to start looking for the adenoma, and intraoperative PTH results suggest when to stop looking. Compared to bilateral exploration Minimal Invasive parathyroidectomy has a smaller incisions, less extensive dissection that leads to reduced postoperative pain and a lower incidence of hypocalcaemia from ischemia of other glands [20,21]. This approach is appropriate for patients with unequivocal imaging suggesting unilateral pathology, no suggestion of concomitant thyroid disease requiring surgical intervention, and no family history of multiple endocrine neoplasia.

When preoperative localization procedures do not identify an adenoma or the adenoma cannot be found during minimally invasive surgery, bilateral neck exploration must be performed. Minimally invasive procedures should be performed by an experienced surgeon in case conversion to a bilateral operation is required. Although there are few randomized trials comparing minimally invasive parathyroidectomy with the traditional bilateral approach, cure rates appear to be similar when the procedures are performed by experienced surgeons. Contraindications for minimally invasive parathyroidectomy include prior extensive neck surgery, hereditary primary hyperparathyroidism, large goiters, multigland disease, obesity, and suspicion of parathyroid carcinoma.

Despite advances in care, subsets of patients with end-stage renal disease still have marked elevations in serum parathyroid hormone levels. Data are lacking regarding whether chronic elevations of PTH in asymptomatic patients warrant parathyroidectomy [22]. Nevertheless, surgery is often performed when patients develop refractory hyperparathyroidism often with serum PTH concentrations above 800 pg /mL [23]. This disorder is called tertiary hyperparathyroidism if hypercalcaemia is present; it is due to a hyperfunctioning parathyroid tissue that does not respond appropriately to physiological regulation or to medical therapy.
with oral calcium salts and calcitriol. The emergence of new therapies, such as calcimimetic agents, may further decrease the need for parathyroidectomy [24,25]. In a combined analysis of four randomized trials, for example, the administration of cinacalcet, compared with placebo, markedly lowered the risk of parathyroidectomy [26]. Several interrelated factors are thought to be involved in the pathogenesis of refractory hyperparathyroidism, namely delayed and/or inadequate therapy, persistent hypophosphatemia and acquired abnormalities of the parathyroid glands, which may be the most important [27]. The autonomous behavior may be caused by reduced expression of the extracellular calcium-sensing receptor and lower vitamin D receptor density in the adenomatous cells [28].

**Surgical Strategies for Parathyroid surgery**

There is lack of randomized controlled trials comparing the long-term effects of medical versus surgical therapy of advanced secondary hyperparathyroidism. Parathyroidectomy in end-stage renal disease is primarily performed in symptomatic patients with markedly elevated and non-suppressible serum PTH values. However, parathyroidectomy may also be indicated for dialysis patients who are asymptomatic but have markedly elevated serum PTH values despite maximal medical therapy with cinacalcet plus calcitriol since some observational studies suggest a benefit of parathyroidectomy among dialysis patients with markedly elevated PTH values [23]. The level at which parathyroidectomy should be considered is not known.

Symptoms that warrant parathyroidectomy in the setting of elevated PTH values are variable and may include those related to hypercalcaemia, hyperparathyroid bone and musculoskeletal disease, and/or calciphylaxis with soft tissue calcifications. Patients with Symptomatic Primary Hyperparathyroidism (PHPT) should have parathyroid surgery [29]. However, many patients with hyperparathyroidism are asymptomatic or have non classical manifestations of their disease. Parathyroidectomy is an effective therapy that cures the disease, decreases the risk of kidney stones, improves bone mineral density, and may decrease fracture risk and modestly improve some quality of life measurements. In addition, proponents of surgery for asymptomatic individuals argue that many untreated patients are lost to follow-up after 5 to 10 years and that the cost of follow-up visits and tests may ultimately exceed the costs of surgery. Thus, some argue that parathyroidectomy is an attractive strategy for nearly all patients, particularly with recent significant progress in techniques for minimally invasive surgical extirpation [30].

Proponents who favor non-operative management for asymptomatic individuals cite the lack of disease progression in the majority of patients and the ability to treat, if necessary, with alternative therapies as reasons to avoid an invasive procedure. Thus, the debate regarding treatment of asymptomatic HPT revolves around the effect of intervention on outcomes, such as symptoms, bone disease, and biochemical abnormalities [31]. However, the specific absolute indications for parathyroidectomy include:

- **Severe hypercalcaemia.**
- **Progressive and debilitating hyperparathyroid bone disease as defined by imaging or histologic evaluation.**
- **Pruritus that does not respond to medical or dialytic therapy.**
- **Progressive extra skeletal calcification or calciphylaxis that is usually associated with hyperphosphatemia that is refractory to oral phosphate binders.** In this setting, PTH-induced release of phosphate from bone contributes to the persistent elevation in
the serum phosphate concentration. Parathyroidectomy will tend to minimize further calcification by lowering the serum calcium and phosphate concentrations.

- Otherwise unexplained symptomatic myopathy.

It is important to appreciate that many of these problems can develop in dialysis patients without significant hyperparathyroidism. Thus, parathyroidectomy should not be performed unless very high PTH levels have been documented. Thus most dialysis patients undergoing parathyroidectomy have a serum intact PTH concentration above 800 pg/mL [23].

There has been a trend to consider surgical intervention earlier in the course of secondary hyperparathyroidism in patients with end-stage renal disease, particularly if the estimated weight of a parathyroid gland exceeds 500 or 1000 mg (normal 30 to 40 mg) [32-34]. This is in part based upon histopathological studies demonstrating that more than 85 percent of glands weighing over 500 mg have nodular hyperplasia [32]. In addition, larger glands may be less likely to respond to medical treatment [35].

Controversy exists regarding the indications for parathyroidectomy in other clinical settings such as anemia that is resistant to erythropoietin which may improve after parathyroidectomy in patients with marked hyperparathyroidism [36]. Possible mechanisms include a direct effect of PTH on erythropoiesis and marrow fibrosis due to significant osteitis fibrosa cystica. However, this type of anemia is also responsive to medical treatment of secondary hyperparathyroidism with intravenous calcitriol. As a result, resistant anemia alone should not be considered an independent indication for surgical intervention [37].

It is also uncertain whether patients with persistent but modest elevations of PTH (between 200 and 800 pg/mL) after intensive medical therapy with calcitriol should undergo parathyroidectomy. Since there are no controlled studies that evaluate the long-term risks associated with persistent moderate hyperparathyroidism, such patients are usually managed medically. However parathyroidectomy is indicated in renal transplant recipients with persistent hyperparathyroidism in association with hypercalcaemia and/or progressive and otherwise unexplained renal insufficiency. Whether parathyroidectomy for severe hyperparathyroidism should be performed prior to renal transplantation is controversial as illustrated by the following observations.

- Once the biochemical abnormalities typical of the uremic milieu disappear after successful renal transplantation, the hyperparathyroidism appears to resolve in most, but not all cases.

- Persistent hyperparathyroidism after transplantation may adversely affect renal function.

- Via unclear mechanisms, there is a risk of abrupt deterioration of renal allograft function after surgical parathyroidectomy [38]. This phenomenon has also been observed in patients with chronic renal failure or end-stage renal disease with residual renal function [39,40]. Therefore the risk of persistent hyperparathyroidism seems to be too small to justify surgical intervention in all patients with severe secondary hyperparathyroidism on the transplant waiting list, since approximately 96 percent appear to do well. On the other hand, potential problems that may arise if parathyroid overactivity persists have to be given serious consideration.

**Parathyroid Surgical Approaches**

Once the patient satisfies the criteria for having a parathyroidectomy, then one of three approaches has to be followed.
• Subtotal parathyroidectomy
• Total parathyroidectomy with autotransplantation
• Total parathyroidectomy

**Subtotal versus Total Parathyroidectomy with Auto-Implantation**

There have been no randomized trials comparing these three techniques, but most surgeons perform either subtotal parathyroidectomy or total parathyroidectomy with autotransplantation. Subtotal parathyroidectomy involves excision of all identifiable parathyroid tissue except for half to one third of the least hyperplastic gland. Drawbacks to subtotal parathyroidectomy include a substantial risk of persistent and/or recurrent disease, which is complicated by greater morbidity if repeat neck exploration is required.

For these reasons, some surgeons prefer total parathyroidectomy with autotransplantation of small amounts of resected parathyroid tissue into the brachioradialis muscle in the forearm. Other sites for autografting such as the sternocleidomastoid muscle, the subcutaneous tissue of the forearm or presternal area, and abdominal fat have also been successfully used. The main advantage of this approach is the ease of removing recurrent hyperplastic glands from the site of implantation under local anesthesia, without the added morbidity of neck re-exploration. Both procedures are extremely successful in controlling the hyperparathyroidism in the short-term; in addition, long-term follow-up also suggests roughly equal proportions of patients with persistent normal PTH levels, recurrent hyperparathyroidism, and permanent hypoparathyroidism [41].

The incidence of reoperation for moderate to severe recurrent hyperparathyroidism is similar with both methods, ranging from 6 to 14 percent [42]. Recurrent hyperparathyroidism is much more likely to occur with autografting of nodular hyperplastic tissue. In two large studies, the frequency of recurrence was 33 percent with nodular versus only 4 percent with diffuse [41,43]. Determining the site of recurrent hyperparathyroidism constitutes a potential disadvantage of total parathyroidectomy with autotransplantation and is often problematic. In one study, the graft was the culprit in only one-half of patients, while hyperplastic tissue found in the neck or mediastinum was responsible for the remainder [44]. Several methods have been used to determine the site of recurrence including:

• The presence of a venous PTH gradient between the arms suggests that the autograft is responsible for recurrent disease, whereas the lack of a gradient suggests that parathyroid tissue remains in the neck or chest.

• Another way to determine graft hyperfunction is to temporarily exclude the graft-bearing forearm from the circulation by using the total ischemic blockade technique [45]. This maneuver produces a “transitory implantectomy” and reliably identifies patients with graft hyperfunction, by showing a 46 to 87 percent reduction in serum PTH after 10 to 20 minutes of arm ischemia.

• Detection of residual or ectopic tissue in the neck may be evaluated by venous sampling, ultrasonography, magnetic resonance imaging, or technetium-99m sestamibi scanning [46].

**Total Parathyroidectomy without Auto-Implantation**

Has the potential theoretical advantage of minimizing the chance of persistent and/or recurrent disease by removing all parathyroid tissue. However, after its institution in the 1960s, it quickly fell out of favor because such patients might develop adynamic bone disease and intractable osteomalacia, permanent hypoparathyroidism, impaired bone healing in the
absence of PTH and its anabolic effects and that they might need long term use of calcium and vitamin D. In spite of these reservations, a majority of patients who undergo total parathyroidectomy have measurable PTH levels at long-term follow-up and no demonstrable bone disease [47-49]. The origin of persisting PTH after total parathyroidectomy is not entirely clear. Missed supernumerary glands could explain a few cases. It is also presumed that small nests of parathyroid cells left behind at surgery undergo hyperplasia because of their continued exposure to the milieu of chronic renal failure. Well designed, randomized, controlled trials comparing the three surgical techniques are lacking, and are unlikely to be performed. Each surgical technique carries its potential advantages and disadvantages. However totals parathyroidectomy without auto-implantation is mostly disfavored by most surgeons, yet currently there are insufficient data to warrant recommendation of one type of procedure over another.

An intraoperative rapid PTH assay has quickly become a popular tool for intraoperative use in all types of parathyroid surgery and for venous localization before surgery. It has been successfully used to guide limited parathyroidectomy in patients with primary hyperparathyroidism [50]. With a turnaround time of less than 15 minutes, the rapid PTH assay works as a biochemical frozen section, providing the surgeon quantitative assurance that all hyperfunctioning parathyroid tissue has been removed. Its role during surgery for secondary hyperparathyroidism has not been well studied; however a non-significant drop in Rapid PTH may indicate a supernumerary or an ectopic gland.

Other parathyroidectomy techniques include percutaneous ethanol injection under ultrasonographic guidance; it has been touted as a safe and simple approach to the treatment of hyperparathyroidism [51,52]. After ultrasonographic identification of the parathyroid glands, ethanol is injected into the largest gland. The serum intact PTH concentration is measured one week after injection [53,54]. If it is still more than 200 pg/mL, ethanol is injected into the same or next largest gland at one-week intervals until the intact PTH level falls to less than 200 pg/mL.

Transient and permanent recurrent laryngeal nerve palsy has been reported with ethanol injection. At present, experience with ethanol injection is limited and the risk of complications uncertain. Ethanol injection holds much promise, particularly if a single large gland is identified or in the treatment of recurrent hyperparathyroidism after subtotal parathyroidectomy. However, this approach should be considered experimental until its efficacy and safety are established in a larger number of patients.

**Pre-requisites for a Safe Parathyroid Exploration**

To avoid surgical complications, the surgeon should review and assess the patient’s medical status as well as the preoperative laboratory and imaging workup. A preoperative checklist is a good way to assure that all safety issues have been addressed. This check list should include:

- Informed consent that includes the possibility of operative failure, transient or permanent paralysis of one or both vocal cords, a change in voice quality and strength, transient or permanent hypoparathyroidism, wound infection, and intraoperative or postoperative bleeding.
- Careful review of the patient’s medical record, check previous laboratory, revise and confirm the diagnosis, based on both clinical and biochemical parameters. Documentation of preoperative vocal cord mobility and any prior neck surgery (e.g. thyroidectomy, an anterior approach to cervical disc repair or tracheostomy).
• All images should be reviewed, and pertinent images should be immediately available to the surgeon in the operating room suite to confirm laterality and location of the abnormal parathyroid gland or glands.

• If intraoperative PTH monitoring is employed, a pre-incision PTH level should be obtained and used as a baseline value. A relative drop in PTH value of >50 percent and into the normal range is suggestive of adequate removal of hyper-functional parathyroid tissue.

• An expert frozen section pathologic analysis should be available.

• If intraoperative recurrent laryngeal nerve monitoring is to be used, the system should be tested prior to prepping and draping the patient.

• Parathyroid surgery is a clean procedure in a well-vascularized area, thus postoperative infections rates are very low. Cefazolin is given selectively for patients who are immunocompromised or with other medical comorbidities.

• Most parathyroid surgeries are performed in the elective setting. Any bleeding diatheses, thrombocytopenia, and platelet dysfunction should be addressed and corrected prior to operative treatment. The risk of cervical hematoma and subsequent airway compromise from parathyroid surgery make it particularly important that all anticoagulants such as warfarin, clopidogrel, aspirin, NSAIDS, and vitamin E are discontinued prior to surgical intervention.

• DVT prophylaxis shall follow the hospital policies in the form of, sequential compression devices, subcutaneous heparin or low molecular weight heparin. Patients should also be encouraged to ambulate before and after surgery.

• Time-out should be performed with the entire operating room team (anesthesiologist, surgeon, and running nurse) before the patient is anaesthetized to assure correct patient identity, laterality of operative field, intended operation, antibiotics, and the informed consent.

**Radioguided Parathyroidectomy**

The use of a radioguided probe has been advocated by some to serve as a useful adjunct in parathyroid exploration. The technique involves intravenous administration of technetium-99m labeled sestamibi approximately two hours preoperatively. Using sestamibi uptake as an indirect measure of parathyroid gland hyperfunction, the surgeon uses a hand-held gamma probe in conjunction with preoperative imaging results to focus the incision over the site of greatest radioactivity. Once the suspected offending gland or glands are removed, intraoperative PTH monitoring is utilized to confirm adenoma excision [55,56].

**Intraoperative Challenges in Parathyroid Surgery**

The challenges of parathyroidectomy include the wide variability in parathyroid gland anatomy between patients the limitations of localization studies, and the possibility of more than four parathyroid glands (supernumerary glands). Accordingly, judicious dissection augmented by thorough knowledge of the wide anatomic and embryologic variations in location is necessary to find an enlarged gland or glands, manage multiglandular disease and deduce the presence of a supernumerary gland [57,58]. The experienced surgeon can accurately recognize size and shape differences among parathyroid glands and reliably estimate their weights [59]. In addition, the surgeon must be able to intraoperatively recognize and properly treat parathyroid carcinoma.
An outline of the general steps and principles to help guide safe and efficient parathyroid surgery includes, general anaesthesia, cervical block anesthesia with monitored conscious sedation for focused parathyroidectomy, positioning by tilting the bed to an angle of approximately 30 degrees in reverse Trendelenburg position, well sited incision, gentle meticulous dissection to follow the natural anatomical neck planes, gentle handling of all suspected to be parathyroid glands, identification of the recurrent laryngeal nerve, and masterly orchestration of the chemical frozen section and the tissue frozen section as well as the timely withdrawal of blood for rapid PTH and the judicious use of the nerve monitor [60,61].

After resection of the first enlarged gland, many surgeons search for the ipsilateral parathyroid gland to support the assessment of a single adenoma versus multiglandular disease and to clarify the anatomic findings. Intra-operative findings need to be documented for future reference in case recurrence or persistence happens.

In primary hyperparathyroidism, a “suppressed” normal gland can be even smaller. Shave or hemi-biopsy of a normal gland is accomplished by gently applying a titanium clip to the distal edge of the gland opposite the vascular pedicle and sharply excising a 5-15 mg fragment, which should then be weighed and assessed by pathology. If the ipsilateral gland is enlarged, and/or if the intraoperative PTH level does not decline appropriately, the diagnosis of multiglandular disease is made, and multigland resection should be performed. Rarely, double adenomas occur on the same side. If an appropriate drop in PTH is documented after the resection of two ipsilateral glands, this may lead the surgeon to be satisfied with a unilateral exploration. In all other cases, bilateral exploration is required, and subtotal parathyroid gland resection is the procedure of choice. Whether bilateral exploration or the use of intraoperative PTH monitoring is being used to identify and manage multiglandular disease, the exploration continues until all enlarged hyperfunctioning glands are identified and dealt with [62]. However, total parathyroidectomy with autotransplantation leads to a higher probability of vitamin D dependence [63].

For patients with familial disease, the amount of parathyroid tissue removed varies with the cause of hyperparathyroidism. For patients with multiple endocrine neoplasia-1 (MEN-1), the initial surgical procedure usually includes resection of three and one-half hyperplastic parathyroid glands (subtotal resection) with a concomitant cervical thymectomy. In patients with MEN-2a, parathyroid hyperplasia is heterogeneous. Initial bilateral exploration is performed, but parathyroidectomy is limited to resection of only the enlarged glands [64].

When a reoperation is required, a more aggressive surgical approach may be employed, which involves complete parathyroidectomy with placement of an autograft, commonly in the forearm. If recurrent hyperparathyroidism develops due to overgrowth of the autograft, it can be treated by graft removal under local anesthesia. However, if the autograft fails to function, the patient may have permanent hypoparathyroidism. The risk of hypoparathyroidism is higher with autotransplantation than leaving the gland in situ [65].

The most common cause for persistent hyperparathyroidism is a missed parathyroid adenoma. During exploration for primary hyperparathyroidism when an enlarged gland is not found in the normal or ectopic positions, despite a very thorough and systematic dissection, the cervical surgical procedure should be concluded. The diagnosis should be reconfirmed, and additional localization studies can be performed postoperatively if reoperation is contemplated. Bilateral jugular vein sampling for PTH levels can be obtained intraoperatively, and used as a localization technique to help clarify whether a missing parathyroid gland is on the left or the right side.
The surgeon must confirm that there is no enlarged gland in the paraesophageal or retroesophageal space, which is the most common place for missed superior glands. Digital palpation along the lateral border of the esophagus is an excellent way to identify the gland, which can be appreciated as a subtle bulge [66]. The missing superior gland may also be found in the direct posterior retropharyngeal space. A median sternotomy should be reserved for reoperation after localization studies have identified a mediastinal gland. The majority of mediastinal parathyroid glands (over 90 percent) are accessible via a cervical approach [67].

For a missing inferior gland or supernumerary gland, the surgeon should explore the ipsilateral thymus and upper cervical region, dissecting into the anterior superior mediastinum via a cervical approach. Missing inferior glands may sometimes be palpated by sweeping a finger from lateral to medial on the peristeum under the manubrium.

Many missing inferior parathyroid glands will be located within the thymus, and most intrathymic enlarged glands can be removed with a cervical approach. Resected thymus should be evaluated with frozen section because a normal or enlarged parathyroid gland may not be readily visible [68].

If exploration is still negative, the carotid sheath is entered sharply and explored to assess for an undescended parathyroid gland. Great care should be taken to avoid damage to the vagus nerve. Exploration of the carotid sheath should extend from the clavicle to the bifurcation of the common carotid. Although rarely indicated, an ipsilateral thyroid lobectomy may be performed if an intrathyroidal parathyroid gland is suspected. About 1 percent of inferior gland adenomas are intrathyroidal [69]. The removal of normal parathyroid glands should be avoided, although only one normal parathyroid gland is necessary for normal calcium homeostasis. If a normal parathyroid gland becomes severely ischemic during dissection or is excised accidentally, the surgeon should send a 1 mm piece of tissue for frozen section analysis and store the remainder of the specimen in ice for expedient autotransplantation.

An Intraoperative Parathyroid Hormone (PTH) level can help to determine whether hyperfunctioning parathyroid tissue has been excised as opposed to normal yet enlarged parathyroid tissue. The PTH level should precipitously decrease with excision of the hyperfunctioning gland. If the PTH level does not drop, then the resected gland is not the cause of excess PTH production. The gland can then be minced and implanted to avoid permanent hypoparathyroidism. Cryopreservation is available in some centers and is useful in some patients to avoid permanent hypoparathyroidism, particularly after reoperative parathyroid surgery. However the need for re-implantation is low at 1% in one study and the success rate was encouraging in the same study and the practice was labeled as questionable [70].

Complications following Parathyroidectomy

Complications following parathyroidectomy are rare, however the patient should be counseled preoperatively about the possible complications, how they present and how they are managed. The complications include but not limited to:

• Failure to achieve durable cure of hypocalcaemia.
• Symptomatic cervical hematoma (0.3 to 1 percent). If it happens a cervical hematoma can cause venous congestion of airway structures, creating significant laryngeal edema, spasm and subsequent airway compromise and eventually obstruction if not well attended to. Therefore postoperatively all patients must be examined to exclude
the presence of a neck hematoma prior to hospital discharge. With rare exceptions
the presence of a symptomatic hematoma indicates urgent surgical evaluation; a
hematoma should never be “observed.” Early recognition and immediate intervention
are crucial to minimize possible mortality [71].

- Hypocalcaemia is an important potential complication. Classically it shows itself within
the first 24 to 48 hours after surgery. It may be transient and mild, due to functional
hypoparathyroidism resulting from suppression of the remaining normal parathyroid
tissue. Symptoms such as perioral or acral paresthesias and anxiety are exacerbated
by hyperventilation. Such symptoms generally respond well to a short course of oral
calcium supplementation. Transient postoperative hypocalcaemia is more common in
patients with severe preoperative hypercalcemia and in those with chronic vitamin
D deficiency (<15 ng/ml). For this reason, preoperative repletion with ergocalciferol
(vitamin D2) (1000 IU per day or more depending on level) may be desirable in vitamin D deficient patients if urinary calcium levels are not elevated. Postoperative
hypocalcaemia can be severe and prolonged resulting in tetany, papilledema, and
seizures. Such patients may require intravenous and oral calcium supplementation
as well as correction of concomitant hypomagnesaemia [72]. Appropriate treatment of
hypocalcaemia will depend in part on phosphate and PTH levels:

- If hypocalcaemia is prolonged and accompanied by hypo or euphosphatemia and high
PTH levels, hungry bone syndrome is diagnosed due to rapid deposition of serum
calcium into demineralized bone following a drop in PTH.

- The predictive factors for the development of hungry bone syndrome are volume of the
resected gland, preoperative blood urea nitrogen concentration, preoperative alkaline
phosphatase level, and old age [73].

- If hypocalcaemia is accompanied by hyperphosphatemia and low PTH levels, hypoparathyroidism is diagnosed, requiring treatment with calcitriol.

- Permanent hypoparathyroidism may occur in patients with previous neck surgery
which can result in resection or devascularization of normal glands. It may also occur
following subtotal parathyroidectomy for multiglandular disease if the remnant is
not viable. If the calcitriol and calcium cannot be tapered off over several months
following surgery, the hypoparathyroidism may be permanent. An undetectable
parathyroid hormone concentration when serum calcium is low can confirm the need
for permanent treatment.

- Recurrent Laryngeal Nerve (RLN) injury is a rare complication in the hands of
experienced parathyroid surgeons and should occur in <1 percent of initial operations.
Preoperative laryngoscopy is recommended in the reoperative setting to assess any
preexisting nerve compromise. Intraoperative neuromonitoring can be helpful in
high risk reoperative cases but does not preclude the need for intraoperative nerve
visualization and meticulous technique [60,74].

- Hyperthyroidism following surgery for primary hyperparathyroidism is an
underappreciated consequence of parathyroidectomy. Biochemical evidence of
hyperthyroidism has been found to occur in 31 to 43 percent of patients following
parathyroidectomy with symptoms of mild thyrotoxicosis presenting in 15 to 27
percent, and is possibly a consequence of thyroid gland manipulation. In general, the
hyperthyroidism is transient and self-limited with normalization of thyroid function
studies within a few weeks to months following surgery. Patients should be monitored
for biochemical or clinical evidence of hyperthyroidism in the early postoperative

179
period. Patients with symptoms can be managed with thionamides and beta-blockade as indicated [75,76].

**Parathyroid Carcinoma**

Parathyroid cancer is a rare endocrine cancer that was first described in 1904 by de Quevain [77]. The incidence of parathyroid cancer has been reported to be 1% to 5% of patients with primary hyperparathyroidism. Parathyroid cancers may occur as sporadic events or as part of syndromes such as Hyperparathyroidism-Jaw Tumor (HPTJT) syndrome, Multiple Endocrine Neoplasia types 1 (MEN-1) and 2A (MEN-2A), and Familial Isolated Primary Hyperparathyroidism (FIHP). The HRPT2 gene is a causative gene for HPT-JT syndrome, and a subset of FIHP patients also has germ-line mutations of this gene [78]. Surgery for parathyroid cancer is en bloc surgical resection of the primary tumor with thyroid lobectomy at the time of the initial operation [79]. The results of radiation therapy and chemotherapy have been described with limited data and conflicting results [80]. At the time of the initial operation, several articles stressed the importance of a resection that includes isthmus, paratracheal, and central neck compartment dissection. However, the clinical usefulness of Prophylactic Neck Dissection (PND) in the management of parathyroid cancer has not yet been established. Some studies recommended en bloc resection along with PND of the central compartment this is thought to be associated with better local disease control and significantly improved long-term survival [81]. An even more aggressive surgical regimen was suggested, that included ipsilateral thyroidectomy, isthmusectomy, skeletonization of the trachea, and excision of the RLN if necessary, thus increasing the risk of operative RLN palsy and postoperative hypocalcaemia. A modified neck dissection should be implemented if cervical lymph nodes are involved. Although most reports suggest that en bloc resection, including thyroid lobectomy with central compartment dissection, is necessary at the time of initial surgery, Sandelin et al. noted that extensive surgery, including PND for patients with parathyroid cancer, did not improve their prognosis [83]. However when the surgeon recognizes a white, firm, fibrous, hypervascular, and/or adherent, enlarged parathyroid gland, this is a parathyroid carcinoma until proven otherwise. The surgeon must recognize this situation to treat it. In general, surgical management includes wide en bloc resection of the tumor and any adherent structures such as the thyroid lobe, with preservation of the recurrent laryngeal nerve unless there is circumferential involvement.

**Summary**

- The current literature contains a lot of controversy and ongoing debate on the investigations, localization techniques and management of hyperparathyroidism with all its classifications.

- Primary Hyperparathyroidism (PHPT) usually presents with subtle symptoms; including polydipsia, polyuria, nephrolithiasis, osteoporosis, fragility fractures, pancreatitis, peptic ulcer disease, gastroesophageal reflux, fatigue, depression, and significant neurocognitive dysfunction. It is diagnosed whenever there is hypercalcaemia detected by routine biochemical screening and an elevated PTH.

- Parathyroidectomy is the treatment of choice for PHPT. It is also performed for all patients with symptomatic and/or familial disease, as well as patients with asymptomatic disease who have decreased glomerular filtration rates, osteoporosis, serum calcium >1 mg/dl above normal, or age less than 50 years. Parathyroid exploration is also indicated for patients with PHPT as a result of parathyroid cancer or parathyroid crisis, and for selected patients with persistent or recurrent primary hyperparathyroidism.
• Patients with familial hypocalciuric hypercalcaemia do not have a primary parathyroid disorder and should not undergo parathyroidectomy.

• Minimally invasive parathyroidectomy techniques combined with intraoperative PTH monitoring have emerged as effective as bilateral cervical exploration for patients who have unilateral pathology as detected by imaging, without thyroid disease, and with no family history of multiple endocrine neoplasia.

• Bilateral neck exploration is indicated:

  • In patients with negative imaging
  • When an adenoma cannot be found with a minimally invasive approach,
  • When the preoperative or intraoperative findings suggest multiglandular disease, for most forms of familial disease, and as directed by concomitant thyroid pathology.

• A missed parathyroid adenoma is the most common cause for a failed initial parathyroid operation and for persistent hyperparathyroidism. During neck exploration for a hyperfunctioning adenoma, understanding the embryology and anatomy of the parathyroid glands is essential to achieve surgical cure.

• Major complications after parathyroidectomy include; failure to achieve durable cure of hypercalcaemia, hematoma with airway compromise, hypoparathyroidism, and recurrent laryngeal nerve injury.

• Hungry bone syndrome is the most common immediate complication of parathyroidectomy, characterized by a precipitous postoperative fall in the plasma concentrations of calcium and phosphorus. Tetany and seizures may occur, possibly leading to major bone fractures.

• Parathyroidectomy is indicated in dialysis patients when they have; severe hypercalcaemia, progressive and debilitating hyperparathyroid bone disease, pruritus that does not respond to medical or dialytic therapy, progressive extra-skeletal calcification or calciphylaxis, or otherwise unexplained symptomatic myopathy in the setting of markedly elevated serum PTH values.

• Parathyroidectomy may also be indicated for dialysis patients who are asymptomatic but have markedly elevated serum intact PTH concentration above 800 pg/ mL despite maximal medical therapy.

• Resistant anemia is not an independent indication for surgical intervention.

• Parathyroidectomy is indicated in renal transplant recipients with persistent hyperparathyroidism in association with hypercalcaemia and/or progressive and otherwise unexplained renal insufficiency. Whether parathyroidectomy for severe hyperparathyroidism should be performed prior to renal transplantation is controversial.

References


