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The Glycoform Modifications of sRAGE Matter

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The signaling of Receptors for Advanced Glycation End Products (RAGE) results in inflammation and tissue remodeling, and has been implicated in several human diseases, including cardiovascular disease [1-3]. Soluble RAGE (sRAGE) encompassing the entire ectoportion of RAGE, but lacking the membrane anchor and cytosolic signaling domain, functions as a decoy that counteracts RAGE-mediated inflammatory signaling by competing for RAGE ligands and dampening the subsequent inflammation and tissue remodeling [4]. RAGE/sRAGE is known to be modified by N-linked glycosylation at two locations of the ligand binding V ectodomain [5], and such modification has been shown to be important for RAGE bioactivity [6,7]. Recombinant sRAGE has been generated and tested in several disease models in mice [8-10] or rats [11], and the results have shown a promise for future clinical applications. However, in these studies, recombinant sRAGE was produced in the fall armyworm (*Spodoptera frugiperda*) cell line sf9 via a baculovirus vector [8-11], rather than in mammalian source, making immunogenicity as well as bioactivity an issue.

Although glycoproteins expressed in insect and mammalian cells are both glycosylated, their glycoforms are different. Insect cells predominantly produce paucimannose N-glycans, whereas mammalian cells can produce highly diverse N-glycoforms including paucimannose, hybrid, and most often, complex type of glycans [12]. These different forms of post-translational modification may impact a therapeutic glycoprotein in the following aspects.

First, glycoforms act as antigens *in vivo*. Insect cell-originated N-glycoforms are immunogenic in mammalian system. Currently, major regulatory authorities (FDA, EMEA) require that therapeutic glycoproteins to be produced in mammalian cell lines such as Chinese Hamster Ovary (CHO) cells and Human Embryonic Kidney cells (HEK293), or in mammalian sources via transgenic animals to avoid adverse side-effects and biosafety concerns [13].

Second, glycoforms contribute to bioactivities or *in vivo* duration of a glycoprotein, directly affecting its therapeutic efficacy [14]. A typical example is recombinant human Erythropoietin (EPO), a therapeutic glycoprotein used for the treatment of anemia-associated diseases. Proper glycosylation significantly enhances EPO bioactivity and duration of action *in vivo* [15]. Other examples include recombinant TNK-tissue plasminogen activator [16], and Soluble Intercellular Adhesion Molecule-1 (sICAM-1) [17].

Although direct evidence regarding N-glycoform modifications and sRAGE *in vivo* efficacy is still lacking, results from several studies have shown that N-glycoform modifications may contribute to RAGE signaling capacity and sRAGE activity *in vitro*. Genetic studies have shown that patients with a G82S polymorphism in AGER (the gene encoding RAGE) are prone to the development of diabetes [6]. Subsequent biochemical studies demonstrated that this polymorphism enhances RAGE N-glycosylation at residue N81, and affects RAGE signal transduction [7]. Since RAGE and sRAGE share the ectodomains, it can be inferred that sRAGE with proper N-glycoform modifications may interact with RAGE ligands more effectively, and thus has higher blocking capacity. Indeed, earlier studies have also shown that sRAGE purified form animal lung tissues is more effective than that of sf9 cell-originated sRAGE to block RAGE ligand-induced vascular smooth cell migration *in vitro*. Recently, Srikrishna and colleagues showed that a small portion of sRAGE from lung tissue, modified by carboxylated N-glycoforms, has higher bioactivity to block NF-κB *in vitro* than that of sRAGE produced in sf9 cells [18]. These studies support the general concept that a proper N-glycoform modification is critical for a glycoprotein's bioactivity and therapeutic efficacy also applies to sRAGE.

Furthermore, the reported dosage of sf9 cell-originated sRAGE is relatively high, i.e. 100 μg/mouse/day for mice [8-10], or 0.5 mg/kg body weight for rats [11]. In addition, either mouse or rat models used for blocking studies required daily sRAGE administration to achieve desired therapeutic outcomes. These suggest that the bioactivity of sf9 cell-originated recombinant sRAGE is relatively low. Significantly, a recent report showing that a similar dose of sf9 cell-originated sRAGE (5 μg/g body weight, assuming an average lab mouse weighs 20 g) elicits monocyte inflammatory reactions in mice [19]. This observation further begs the question of whether it is apt to use sf9 cell-originated, rather than the mammalian cell-originated sRAGE with proper N-glycoform modifications in animal studies.

In our opinion, the specific N-glycoform modification of sRAGE matters. Systematic studies of the bioactivity and assessment of the *in vivo* efficacy of mammalian cell-originated recombinant sRAGE in animal models will test this concept. Information gleaned from these studies will render a more adequate assessment of sRAGE's therapeutic potential, including possible reduction of administrated dosage and administration frequency, owing to higher bioactivity and/or *in vivo* efficacy that are resulted from using a proper N-glycoform modified form. These studies will also provide a basis for further development of sRAGE as a candidate pharmacological glycoprotein for future clinical applications.

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