Sugar signals of proteoglycans in neurobiology and tumor biology

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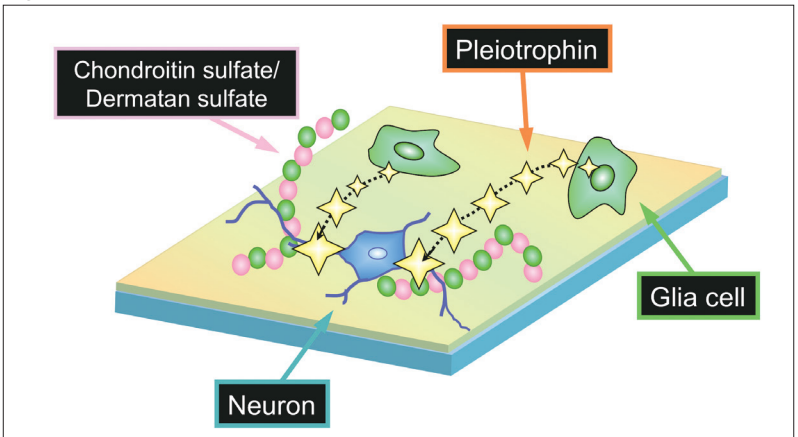
Glycosaminoglycans (GAGs) side chains of proteoglycans at cell surfaces and extracellular matrices play critical roles in various biological processes including cell proliferation, differentiation, tissue morphogenesis, brain development, infection, inflammation, malignant transformation, and metastasis of tumors cells. We have been studying functions of GAG sugar chains in brain development and tumor metastasis, and have demonstrated the critical roles of the sugar sequences during the brain development and the metastatic behavior of tumor cells in mouse systems.

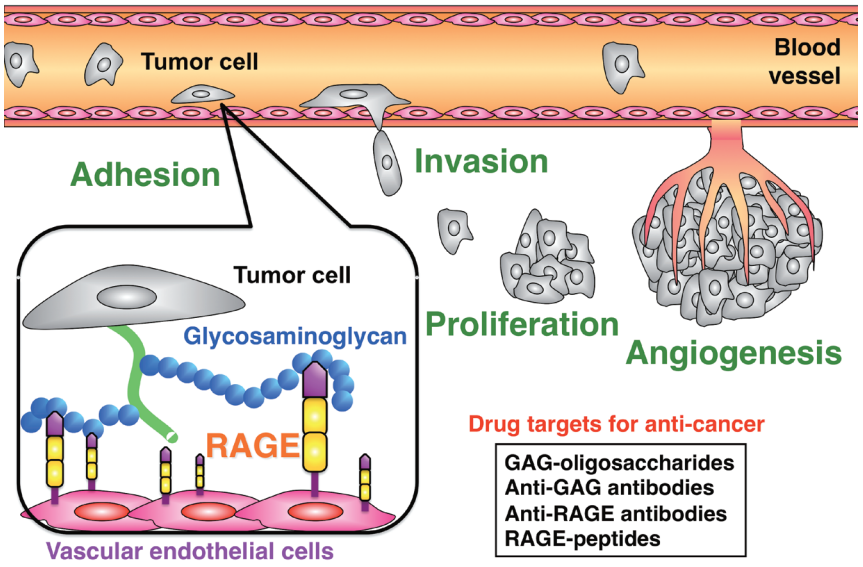
In neurobiological studies we have demonstrated that chondroitin sulfate (CS) / dermatan sulfate (DS) hybrid chains of embryonic pig brain unlike those from adult pig brain exhibit neurite outgrowth promoting activity towards embryonic mouse hippocampal neurons in culture [1], and that this activity is mediated through binding to a neurotrophic factor, pleiotrophin [2](**Fig. 1**). A series of sulfated octasaccharide sequences containing D-disaccharide units [GlcUA(2-*O*-sulfate) β1-3GalNAc(6-*O*-sulfate)] have been isolated after digestion with chondroitinase B, which specifically cleaves IdoUAα1-3GalNAc(4-*O*-sulfate) linkages in CS/DS hybrid and DS chains [3].

Fig. 1



Molecular Mechanism of Neurite-outgrowth Promoting Activity of Embryonic Brain-derived Chondroitin sulfate/Dermatan sulfate Hybrid Chains. The Chains Exhibit their Activity through Specific Capturing and Presenting Pleiotrophin Secreted from Glial Cells towards Embryonic Hyppocampal Neurons.

Fig.2



Involvement of GAGs and RAGE in the Pulmonary Metastasis of Tumor Cells. Glycosaminoglycans Expressed on Tumor Cell Surfaces are Recognized by RAGE Presumably Expressed on the Vascular Endothelium.

In addition, important roles of E-type CS chains containing E-disaccharideunits[GlcUAβ1-3GalNAc(4,6-*O*-disulfate)] in the development of mouse brain have also been demonstrated [4] using *in situ* hybridization of a sulfotransferase, which synthesizes the E-type structure and immunostaining with a specific phage display antibody specific for chondroitin sulfate E-type (CS-E) and can recognize a series of structurally defined CS-E decasaccharide sequences [4)5)]. Based on these and other studies we have proposed the "Wobble hypothesis" for the sugar sequences involved in biological processes where multiple distinct sequences interact with a single functional protein [6].

In recent tumor biological studies, a higher expression of the CS-E epitope consisting of E-disaccharide units was demonstrated in highly metastatic Lewis lung carcinoma (LLC) cells as compared with low metastatic LLC cells. The metastasis was dramatically inhibited in a mouse system by enzymatic removal of CS chains from the tumor cell surface, pre-injection of CS-E in a dose-dependent manner or pre-injection of a phage display antibody specific for CS-E [7]. These findings prompted us to investigate the role of the CS and/or GAG structure of the tumor cells in the experimental lung metastasis.

More recently, to characterize CS-E-binding proteins in a mouse lung, a detergent extract of a mouse lung homogenate was analyzed by affinity chromatography using a CS-E-immobilized column [8]. A CS-E-binding putative receptor was identified as Receptor for Advanced Glycation End-products (RAGE) by SDS-PAGE followed by MALDI-TOF-MS of the trypsin digest of a protein band, suggesting that the E-unit-containing epitopes of CS chains. Furthermore, heparan sulfate chains at the tumor cell surfaces were also recognized by RAGE. Thus, RAGE and GAGs are promising targets for diagnosis and therapy of malignant tumors (**Fig. 2**).

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