Panel Discussion

Biological functions of glycosaminoglycans

Shoko Nishihara

Department of Bioinformatics,
Graduate School of Engineering,
Soka University, Japan

Professor Shoko Nishihara received her Ph.D. in 1982 from Dept. of Chemistry, Graduate School of Science, the University of Tokyo. She continued her research as a research associate at Dept. of Microbiology, the Jikei University School of Medicine. Then she worked on *Drosophila* oocyte maturation as a postdoctoral fellow at Dept. of Biochemistry, University of North Carolina (Prof. Laura Kalfayan) and continued her *Drosophila* work as a postdoctoral fellow at Dept. of Molecular Biology, Mitsubishi Kasei Institute of Life Sciences. In 1991 she moved as an assistant professor to Institute of Life Science, Soka University (Prof. Hisashi Narimatsu) and was introduced to the field of glycobiology. In this period she contributed to the molecular cloning and characterization of the fucosyltransferase gene family. In 2001 she moved to her current position, Professor, at Dept. of Bioinformatics, Graduate School of Engineering, Soka University. She is a member of the Editorial Board of the Glycoconjugate Journal. Research projects within her group are focused on analysis of glycan functions in embryonic stem cells, analysis of glycan functions by *Drosophila* model system and the functional analysis of PAPS transporters.

Introduction

Proteoglycans are composed of negatively charged glycosaminoglycans (GAGs) and core proteins. GAGs include heparan sulfate (HS), chondroitin (CS)/dermatan sulfate (DS), keratan sulfate (KS) and hyaluronan (HA). Various growth factors, morphogens and cytokines interact with GAGs, which regulate the signaling pathways of these factors. Therefore, GAGs have important roles in various developmental processes such as embryogenesis, morphogenesis, neurogenesis and immunogenesis, in addition to progressions of diseases including cancer and hereditary disorder.

Heparan sulfate and chondroitin sulfate are conserved across a wide range of species

The glycosylation of proteins and lipids is performed in the Golgi apparatus by various glycosyltransferases. Molecular evolutionary study about glycosyltransferases showed that the divergence of gene families had finished before the branching of the deuterostome and protostome such as *C. elegans* and *Drosophila melanogaster* [1]. After that, early in the history of the vertebrate lineage, intrafamilial genes increased through gene duplication to obtain a variety of substrate specificities for synthesizing various glycan structures. A prototype set of glycosyltransferases including HS- and CS-synthesizing enzymes existed before the protostome-deuterostome split, suggesting conservation of these GAG structures

and functions from *C. elegans* and *Drosophila* to mammals. However, DS, KS and HA could not be found in *C. elegans* and *Drosophila*. Whole body or tissue-specific knockdown (KD) of 125 *Drosophila* glycosylation-related genes were performed [2]. Whole body gene KD of HS- and CS-related genes resulted in lethality before eclosion. However, sulfotransferases showed lower ratio of lethality than glycosyltransferases synthesizing HS and CS backbone structure. The tissue-specific KD of HS- and CS-related genes showed the highest ratio of abnormalities in the corresponding tissue among all glycosylation-related genes, indicating that HS and CS were essential for development. The similar malformed phenotypes were observed between HS-related and CS-related genes, suggesting that they regulate similar signaling pathways.

Biological functions of heparan sulfates

HS proteoglycans (HSPGs) or CS proteoglycans (CSPGs) are composed of negatively charged HS or CS and core proteins. There have been many studies of the biological functions of HSPGs in *Drosophila* [3),4)]. They play important roles in the regulation of fundamental signaling pathways; for example, Hedgehog (Hh), Decapentaplegic (Dpp; a bone morphogenetic protein (BMP)-type ligand that belongs to the TGFβ family), Wingless (Wg), fibroblast growth factor (FGF) and Slit-Roundabout (Slit-Robo) signaling. Hh, Dpp and Wg, which are secreted signaling molecules, function as morphogens during various stages of development. HSPGs bind to them, stabilize them, determine their distribution, probably work as co-receptors, and then regulate the expression of downstream target genes of their signals. HSPGs also bind to FGFs and work as co-receptors. In addition, HSPGs bind to major axon guidance cues, such as Slit and Netrin, and determine their distribution. Thus, HS, rather than core protein, binds to various ligands and regulate their signals in many aspects of development in *Drosophila*.

The fundamental functions of HS are conserved in mammals. Again, HS binds to Hh, BMP, Wnt, FGF, and so on to regulate their signals [5)]. In early mouse embryogenesis, HS on the surface of extra-embryonic endoderm (ExE) cells are essential for the stable and local retention of FGF ligands to the FGF receptor and subsequent activation of FGF signaling required for ExE development [6)]. In mouse embryonic stem (ES) ells, HS also regulates the important extrinsic signals for their maintenance and differentiation (**Figure 1A**) [7)]. HS is present at higher levels than other GAGs such as CS/DS and HA on mouse ES cells, and the levels of each GAG increases during differentiation. HS contributes to the maintenance of the undifferentiated naïve state of mouse ES cells through BMP/Smad signaling and probably through Wnt/β-catenin signaling. BMP4 and Wnt3a bind to heparin (K_D = 69.4 nM and 26.0 nM, respectively). However, the situation of Wnt signaling is bit complex, and not yet resolved [7)]. The

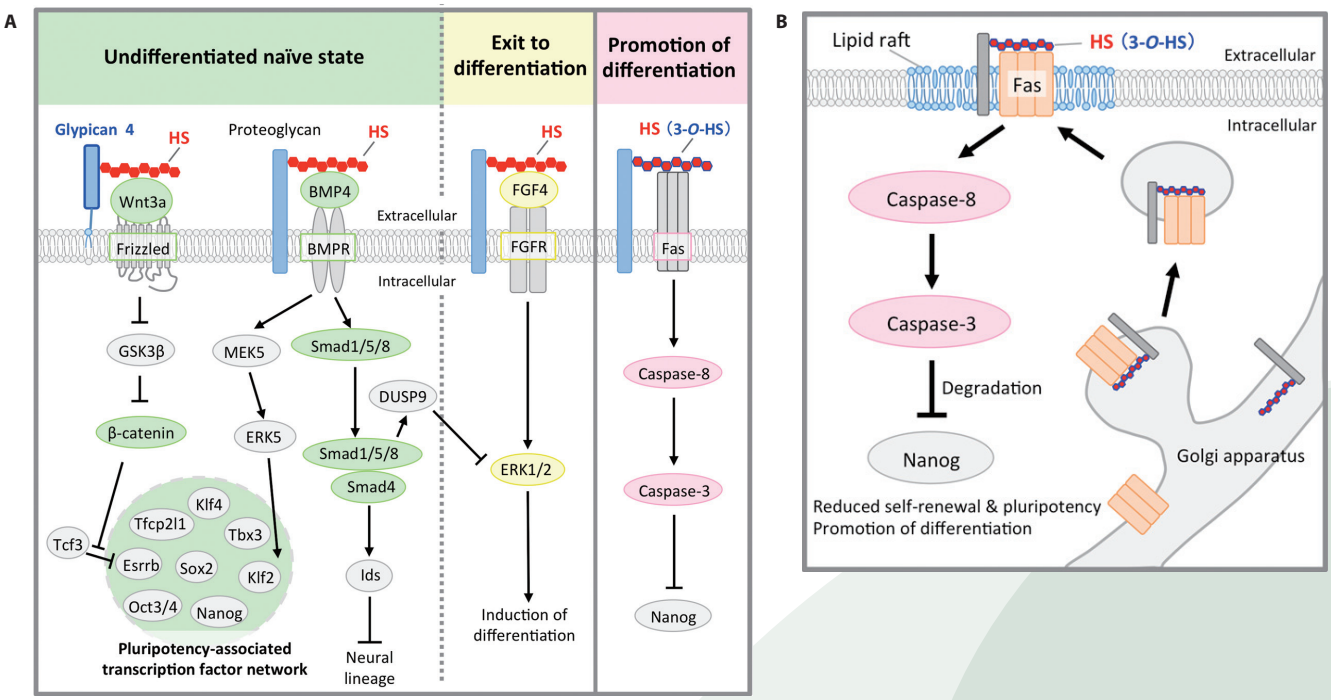

Figure 1

Overview of heparan sulfate (HS) functions in undifferentiated naïve mouse ES cells and during differentiation from ES cells.

A. HS contribution to the signaling events triggered by extrinsic factors.
The undifferentiated naïve state of mouse ES cells is regulated by many extrinsic factors. HS contributes to the maintenance of this state through Wnt/β-catenin and BMP/Smad signaling, and to the exit from this state through FGF4/ERK signaling. HS proteoglycan Glypican 4 specifically regulates Wnt/β-catenin signaling. 3-*O* sulfated HS (3-*O*-HS) promotes to the differentiation from the undifferentiated naïve state through Fas signaling.

B. Mechanism of Fas signaling *via* 3-*O*-HS in promotion of differentiation. During the differentiation from the undifferentiated naïve state, 3-*O*-HS are upregulated, and then bind to Fas. They translocate intracellular Fas into lipid rafts on mouse ES cell surfaces. The accumulation of Fas in the lipid rafts activates the Fas/caspase-8/caspase-3 signaling cascade, and the subsequently activated caspase-3 degrades the Nanog protein, which is a key transcription factor for maintaining self-renewal and pluripotency of ES cells.

contributions of HS to Wnt signaling may depend on core proteins. For example, HS on proteoglycans other than Glypican 4 may act as a reservoir, inhibiting binding of Wnt to cognate receptors, whereas HS on Glypican 4 may act as a co-receptor or a stabilizer. In addition, HS contributes to exit from the naïve state *via* FGF4 signaling, and then 3-*O* sulfated HS promotes the differentiation through Fas signaling from naïve mouse ES cells into primitive endoderm or primitive ectoderm and the subsequent three germ layers (**Figure 1B**).

Biological functions of chondroitin sulfates

Some studies have been done for the function of CSPGs in *C. elegans*. There is only one chondroitin synthase sqv-5 in *C. elegans*, of which mutant showed squashed vulva phenotype. Whole body KD by using synthesized double-stranded RNA of *sqv-5* proved that Chn, non-sulfated forms of CS, is involved in embryonic cell division, namely cytokinesis and chromosome partition [8]. Cytokinetic regression caused by loss of CS is also observed in the embryo of *GlcAT-I* knockout (KO) mouse, indicating that the Chn/CS function in embryonic cell division is conserved from *C. elegans* to mammals. In addition, *GlcAT-I* KO ES cells demonstrated that CS, probably CS-A and CS-E, promotes initial ES cell commitment to differentiation *via* binding to E-cadherin. Furthermore, CS-E shows strong binding affinity to Wnt3a (K_D = 27.3 nM), comparable to heparin, as well as various growth factors, neurotrophic factors and cytokines *in vitro* [9]. During differentiation from ES cells to cardiomyocytes, CS-E inhibits Wnt signaling, leading to a reduction in early cardiac progenitors and an enhancement of later differentiation steps into mature cardiomyocytes.

CSPGs and CS-E are also involved in neuronal plasticity [9),10),11)]. Perineuronal nets (PNNs) are a specialized extracellular matrix surrounding parvalbumin-positive interneurons, a subclass of GABAergic inhibitory neurons. PNNs in adult consist of CSPGs, HA, tenascin R, Otx2 and Semaphorin 3A, which strongly interacts with CS containing CS-E and inhibits neurite outgrowth. Transgenic mice overexpressing *C6ST-1* inhibited the formation of adult 4-*O*-sulfation-enriched PNNs and showed ocular dominance plasticity even in the adult mice, indicating that 6-*O*-sulfated CS is essential for neuronal plasticity. After CNS injury including spinal cord injury, functional recovery is difficult because of glial scar formation, which inhibits axon regeneration [10),11)]. Reactive astrocytes secrete extracellular matrix proteins including CSPGs and KS proteoglycans (KSPGs), and then they form a glial scar. In this case, CSPGs and KSPCs inhibit axon regeneration, while HSPGs promotes it, because of the different type of binding to receptor protein tyrosine phosphatase sigma.

Conclusion

As mentioned above, GAGs bind to morphogens and growth factors to regulate their signals as co-receptors. In addition, GAGs directly bind to GAG receptors as ligands to regulate downstream signals. These signals work as key regulators for many biological processes including development, although I covered just several examples of their functions here. Therefore, the defects of GAG-related genes induce many types of hereditary diseases such as Ehlers-Danlos syndrome, bipolar disorder and hereditary multiple exostoses. When we are faced with the challenges of GAG functions, we should consider the following two points: 1) sulfation pattern of GAG and 2) core-protein, though it may not be identified easily. The former determines the binding specificity to each signal molecule and the latter controls GAG localization, which may determine whether GAG acts as a co-receptor or as a reservoir, namely inhibiting binding of ligands to cognate receptors. GAGs also bind to cytokines and chemokines and regulate their signals, indicating their functions in immunogenesis, inflammation and also cancer progression. HA in the tumor microenvironment modulates the function of tumor-associated macrophages to support self-renewal of cancer stem cells. It is worth mentioning that many viruses and other pathogens bind to GAGs on cell surface and use them as entry sites of infection. Thus, GAGs have huge variety of functions in a wide range of biological and medical fields, and therefore, the applications of GAGs based on their functions will be spread into various arenas including regenerative medicine and cancer therapy.

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