

Heparan Sulfate Proteoglycans on the Plasma Membrane; Association with Detergent-resistant Membranes

Katarzyna A. Podyma-Inoue, Miki Yokoyama, Tomoko Kimura and Masaki Yanagishita
Dept. of Hard Tissue Engineering, Biochemistry, Tokyo Medical and Dental University

Heparan sulfate proteoglycans (HSPGs) are widely present on the surface of animal cells. They have an ability to bind to and regulate a number of extracellular ligands. Cell surface HSPGs are strategically located so they are used for intercepting and regulating biological signals coming into cells. Mechanisms involved in controlling the localization and number of HSPG molecules on the cell surface could play important roles in regulating functions of biologically active molecules. Recently, the localization of some HSPGs to the lipid rafts, specialized cholesterol- and glycosphingolipid-rich plasma membrane domains involved in signal transduction has been suggested. Using metabolic radiolabeling experiments and sucrose-density gradient ultracentrifugation, we have identified ³⁵S-labeled macromolecules associated with detergent-resistant membranes isolated from a rat parathyroid (PTr) cell line. The low-density membrane fraction contained at least 4-5% of the total ³⁵S-labeled macromolecules, implying specific recruitment of HSPGs to the lipid rafts. Identity of ³⁵S-labeled molecules as HSPG was also confirmed by Western blotting using antibodies recognizing ΔHS-derived epitope. Analyses of core proteins revealed a major band at 30-33 kDa and minor one, at 75 kDa. The major band was identified as syndecan-4, while the minor one with 75 kDa MW corresponded to syndecan-1, suggesting the presence of different HSPG-associating membrane domains. The HS chains seemed to be important for the structural integrity of lipid rafts since the heparitinase treatment altered the constituent proteins in intact DRMs. On the other hand, core protein of HSPGs was retained in detergent-resistant membranes even after heparitinase treatment, implying that HS chains were not indispensable for the recruitment of HSPGs to membrane domains.

Although our studies demonstrated that syndecan-4 was a predominant HSPG species in DRMs in PTr, the presence of other HSPGs, such as glypicans has to be also considered. Detailed biochemical investigation

of HSPGs present on the cell surface will help us to define the **molecular** nature of HSPG-ligand interactions. Further studies, involving proteomic characterization of **HSPG-containing membrane domains will reveal** the proteins possibly interacting with HSPGs in the lipid rafts and will provide critical information on the nature of signaling/endocytic complexes.

... is likely to be involved in several biological phenomena
... metastasis and angiogenesis
... heparanase encodes endo- β -D-glucosaminidase
... overexpressed in many human tumor types, such as those in the head
... hepatocellular carcinoma, esophageal
... and cultured human tumor cell lines, such associations are
... give to indicate the involvement of heparanase in tumor progression
... able to develop anti-metastatic drugs against tumors expressing
... heparanase, it will be necessary to fully understand the activation
... programs of heparanase. We have demonstrated that i)
... expression of heparanase in tumor cells is due to the abundant DNA
... methylation, ii) six N-glycosylation sites are necessary for the
... heparanase secretion, and iii) a disulfide bond is required for the
... heparanase activation. Thus, these present findings will provide a basis
... development of novel heparanase inhibitors
... furthermore, we have screened for small-molecule heparanase
... inhibitors from 10,000 microbial proteins of actinomycetes, fungi, bacteria
... and synthetic compounds. We obtained 4-Bn-RK-882 as a
... heparanase-specific inhibitor. 4-Bn-RK-882 inhibited both tumor
... invasion and metastasis *in vitro* and *in vivo*.