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

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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 ^{35}S-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 ^{35}S-labeled macromolecules, implying specific recruitment of HSPGs to the lipid rafts. Identity of ^{35}S-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.