Cell Surface Heparan Sulfate Proteoglycans: Selective Regulators of Ligand-Receptor Encounters*

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Cell surface heparan sulfate proteoglycans (HSPGs),1 substantially more abundant than most receptors, modulate encounters of extracellular protein ligands with their receptors by forming HS-protein complexes. Two gene families account for most cell surface HSPGs. Both consist of discrete core proteins covalently attached to two or three chains of HS, an N- and O-sulfated linear polysaccharide of repeating disaccharides containing N-acetylgalactosamine (GlcNAc) and uronic acid (glucuronic acid (GlcA) or iduronic acid (IdoUA)). The syndecan family was the first discovered, which in mammals contains four gene products with distinct extracellular domains (ectodomains) and highly conserved short cytoplasmic domains. These apparently extended proteins place the HS chains distal from the plasma membrane (1, 2). The syndecan family contrasts with the glypican family, which in mammals contains six gene products that are covalently linked to plasma membrane lipid by glycosylphosphatidylinositol anchor (1, 3). The glypican core proteins contain six invariant disulfide bonds, are likely to be globular, and place HS chains adjacent to the plasma membrane. Expression of both the syndecans and glypicans is extensively regulated during mouse embryogenesis and results in discrete adult expression patterns for each HSPG such that every adherent cell exhibits a distinct repertoire of cell surface HSPGs.

Binding to HS chains is remarkably widespread among extracellular proteins, especially matrix proteins, proteases and their inhibitors, lipases, lipoproteins, growth factors and their binding proteins, cytokines, chemokines, collectins, and antimicrobial peptides. These proteins are involved in morphogenesis, tissue repair, energy balance, and host defense (Fig. 1). Additionally, numerous pathogens (e.g. herpes simplex virus, Neisseria, Plasmodium) bind to the cell surface via HS (4). Importantly, many of these ligand-HS interactions are mostly modulating or regulatory and do not typically result in cellular signaling from the HSPG. The best characterized of these interactions is the recognition of a specific pentasaccharide sequence by antithrombin III (9). FGF-2 binds most tightly to a specific hexasaccharide sequence, but an additional 4–6 sugar residues are required to activate the receptor (10). Specific oligosaccharide binding sequences are known for multiple ligands (11–14); however, there is no universal consensus amino acid sequence for protein binding to HS chains. Most studies suggest that multiple arginine and/or lysine residues aligned on the protein surface accommodate a distinctive array of anionic sites on the HS chain (14).

HSPGs Regulate Ligand-Receptor Encounters as Coreceptors

Cell surface HSPGs bind to a large number of proteins; thus, some of their molecular interactions have been considered nonspecific. This perception is challenged by both in vivo evidence showing that cell surface HSPGs are required for specific morphogenetic events and by a growing body of data demonstrating that HS interactions with ligands depend on specific HS sequences. These interactions are mostly modulating or regulatory and do not typically result in intracellular signaling from the HSPG. The studies reviewed below suggest that when HSPGs accelerate ligand-receptor encounters, the subsequent receptor action depends on whether the ligand is soluble or insoluble.

Soluble Ligands—Extensive research has focused on HSPGs as coreceptors for a large variety of soluble ligands, including FGFs, transforming growth factors-β1 and -β2, vascular endothelial growth factor (VEGF165,166), CC and CXC chemokines, and various
cytokeratins (1). The diverse cognate receptors for these ligands include receptor tyrosine kinases and seven pass G-protein-coupled receptors. In this role, the HSPGs can modulate ligand-receptor encounters by altering ligand concentrations, stability, or conformation and by ligand or receptor oligomerization. Indeed, there seem to be several distinct mechanisms by which the HSPGs act as coreceptors.

One of the best studied examples is that of the fibroblast growth factor receptor-1 (FGFR-1), a receptor tyrosine kinase, and its cognate ligands FGF-1 or -2. Rapraeger et al. (15) first demonstrated that in the absence of cell surface HS, FGF-2 interacts poorly with its cognate receptor and does not activate downstream intracellular signaling. Surprising studies showed that HS, FGF-2, and FGF-1 form a ternary complex at the cell surface, where HS can be provided by either syndecans or glypicans (16). The HS in the complex solely accelerates the ligand-receptor interactions, as is evident from the finding that its absence can be overcome by increasing either reactant concentration. Because FGF-1 must dimerize to signal, the cell surface HS is thought to bind monomeric FGF-2 and form oligomers that, in turn, dimerize the receptor (5, 6). Despite its simplicity, this model is controversial. For example, it was recently shown that the minimal subunit for FGF-1 activation is a monomer, suggesting that the HS provides a template for the FGF-2 and FGF-1 interaction and does not act by oligomerizing either ligand or receptor (17). Similarly, for the FGF-7 receptor, heparin or HSPG-induced oligomerization of either FGF-1 or -7 does not correlate with biological activity, supporting the model that HSPG-induced ligand oligomerization is not critical in formation of the receptor-ligand complex (18).

Insoluble Ligands—Binding to insoluble ligands immobilizes the HSPG in the plane of the membrane, which causes the syndecan cytoplasmic domain to interact with the actin cytoskeleton and form more stable adhesions. Because glypicans are linked only to the outer membrane leaflet, their direct interactions with cytoplasmic elements are limited. Individual syndecan cytoplasmic domains do not produce soluble cytoplasmic signals, but they can oligomerize, be phosphorylated, and interact with scaffolding and signaling molecules (19–21).

The short syndecan cytoplasmic domains (28–34 amino acids) contain three functional subdomains, C1, V, and C2, at the C terminus. The highly conserved C1 subdomain contains binding sites for Src protein tyrosine kinases (21). The variable V subdomain of syndecan-4 can interact with phosphatidylinositol bisphosphate and the catalytic domain of protein kinase C, interactions thought to result in oligomerization of the cytoplasmic domain and localization of syndecan-4 to focal adhesion complexes (22). A recently identified intracellular protein, syndesmos, also binds specifically to the cytoplasmic domain of syndecan-4 via the C1 and V subdomains (23). This interaction can regulate cell spreading and actin stress fiber organization.

The C2 subdomain contains a conserved amino acid sequence, FYA, which is recognized by type II PDZ domain binding proteins. Syntenin and CASK, PDZ domain proteins that form subplasmalemmal scaffolding, bind immobilized syndecan cytoplasmic domains in vitro, co-localize with syndecans at the plasma membrane in cells, and appear to link the syndecans and the cytoskeleton. Syntenin is a widely distributed protein comprising an N-terminal region of unknown function followed by two tandem PDZ domains, resulting in a 1:2, syntenin-syndecan molecular stoichiometry (24, 25).

CASK binds to the C2 subdomain in the syndecans with its single PDZ domain (26). CASK also contains a Ca2+/calmodulin kinase and an enzymatically inactive guanylate kinase domain (27) that can bind to the T-box transcription factor, Tbr-1. Importantly, when CASK and Tbr-1 are coexpressed, CASK translocates to the nucleus where it acts as a coactivator for this transcription factor (28). However, overexpression of syndecan-3 results in retention of CASK with syndecan-3 in the perinuclear endoplasmic reticulum, reducing its translocation to the nucleus. This result was unexpected because syndecan-3 would be expected to localize to the cell surface. Nonetheless, these findings imply a link between syndecan interactions at the cell surface and transcriptional activity in the nucleus.

These interactions of the cytoplasmic domains and possibly other yet-to-be-established are responsible for the effects of syndecans on cell shape and adhesion. Syndecan-4 participates with integrins and a variety of kinase-based signaling systems in the formation of focal adhesions (22, 29). Syndecan-1, which polarizes to the basolateral surfaces of epithelial cells, is required for the maintenance of epithelial morphology and organization (30).

Several homologues of vertebrate HS biosynthetic enzymes have been identified in Drosophila by genetic screens for functional mutants of Wingless signaling and of the secreted morphogens FGF, Hedgehog (Hh), and/or Wg (31) (Table I). Genetic analyses implicate daily in signaling by both Wingless (wg, a Wnt family member) and Decapentaplegic (dpp, a transforming growth factor-β family member). The data are consistent with Dally acting as a coreceptor for these morphogens, yet there is unexplained selectivity in the effect of the mutation; Dally affects Wg activity in the embryo, but Dpp activity is affected solely in imaginal discs and not earlier during development. The basis of this selectivity is unclear.

Several homologues of vertebrate HS bio-synthetic enzymes have been identified in Drosophila by genetic screens for functional mutations in signaling mediated by the secreted morphogens FGF, Hedgehog (Hh), and/or Wg (31) (Table I). These putative enzyme genes are UDP-glucose dehydrogenase (sugarless, sgl), HS copolymerase (toul velu, ttv), N-deacetylase/Sulfotransferase (NDST) (sulfateless, sfl), and 2-O-sulfotransferase (pipe). The enzymatic activity of sgl has been demonstrated (32) but has not been assessed for ttv and sfl. However, sgl, sfl, and ttv mutant flies synthesize abnormal HSPGs that correspond structurally to the expected enzyme deficiency (33, 34).

Other Drosophila mutations that can ablate the role of HSPGs as coreceptors in the action of FGFs (46). Two identified FGF receptors are associated with alterations in mesodermal migration and cardio genesis (Heartless, Htl) and in branch formation in the tracheal system (Breathless, Btl) (35, 36). Mutations in the FGF ligand (Branchless, Bnl) recapitulate the Btl mutation (37). Importantly, sgl and sfl show similar phenotypes (38). As expected, FGF-dependent MAP kinase activation, the downstream target of Btl and Htl, is reduced in sgl and sfl mutants. Furthermore, consistent with the role of HSPGs as FGF coreceptors, a constitutively active form of the receptor partially rescues mutations in both sgl and sfl, and overexpression of Bnl can partially overcome the requirement of sgl and sfl in Btl mutants.

The selective coreceptor functions of HSPGs are conserved in mammals. Transgenic mice expressing the wnt-1 oncogene driven by the mouse mammary tumor virus long terminal repeat develop mammary alveolar hyperplasia and frequent mammary adenocarcinomas. The hyperplasia and tumorogenesis are abolished when these mice are made syndecan-1-deficient by crossing with syndecan-1 null mice (39). Thus, syndecan-1, the predominant HSPG in mammary epithelial cells, apparently acts as a coreceptor for...
kinase pathway (44). Other signaling pathways also appear to belong to the TIMP-3-sensitive ADAM (a disintegrin and metalloproteinase) family. These properties suggest that shedding of syndecan-1 ectodomains is accelerated by tissue injury, the enzyme digests the NA domains. This releases the enzyme-resistant NS domains that now activate FGF-2 mitogenicity (48). Indeed, purified syndecan-1 ectodomains can also function as potent inhibitors of heparin-mediated FGF-2 mitogenicity via the NA domains of their HS chains (44). However, when the same syndecan-1 ectodomains are treated with platelet heparinase, as would happen during tissue injury, the enzyme digests the NA domains. This releases the enzyme-resistant NS domains that now activate FGF-2 mitogenicity (48). Indeed, mitogenically active concentrations of the heparin-like NS domains are generated in HS biosynthetic enzymes and proteoglycan core protein genes

| HS biosynthetic enzymes | Mutant gene | Phenotype |
|-------------------------|-------------|-----------|
| UDP-N-acetylglucosamine 6-deacetylase | Sugarless (sgl) | Segmental patterning, mesodermal migration, and cardiac defects |
| GlcNAc/GlcA copolymerase | Taut (sgt) | AP wing patterning defect |
| N-Deacetylase/ N-sulfotransferase | Sulfatless (ssf) | Same as sgl |
| 2-O-Sulfotransferase | Pipe (60) | DV patterning defect |
| Prostaglycan | Daily (61, 62) | Segmental axial patterning defect |
| Glypican | | |
| Syndecan | Deyo (63) | |

| Mammal | Mutant gene | Phenotype |
|--------|-------------|-----------|
| Ext 1 and 2 | Multiple exostoses (benign bone tumors) |
| NDST-1 | Nonviable, immature lungs at birth |
| NDST-2 | Absent mast cell heparin |
| 2-OST-1 | Absent kidneys; eye, skeletal defects |
| Gpc-3 | Somatic overgrowth, distinctive faces, multiple anomalies (Simpson-Golabi-Behmel syndrome) |
| Sdc-1 and -4 | Viable, fertile, no anomalies |

Mutations identified in HS biosynthetic enzyme and proteoglycan core protein genes

| Mutant gene | Phenotype |
|-------------|-----------|
| Mutant gene | Phenotype |

Shed, Soluble HSPGs Regulate Molecular Encounters in the Extracellular Space

Cell surface HSPGs are released from the cell surface in a process commonly known as shedding (40, 41). Shedding of cell surface HSPGs was thought to occur only as part of normal turnover for these molecules. However, recent data indicate that shedding can also be a highly regulated cellular response to biological cues and that shed ectodomains themselves act as regulators of molecular encounters.

Mechanism of Shedding—Cell surface molecules are shed when they are cleaved by a family of enzymes known collectively as sheddases or secretases, and their ectodomains are released relatively intact from the cell surface (42, 43). Shedding is an important mechanism of activation and secretion for approximately 1% of cell surface proteins, including growth factors, cytokines, cell adhesion molecules, and enzymes, among others. Because the shed ectodomains of these molecules perform critical functions in various pathophysiological events such as septic shock, cell proliferation, and host defense, the process of shedding has been targeted for prophylactic and therapeutic interventions, and the mechanism has been under intense investigation (43).

Each of the four mammalian syndecans is shed rapidly from the cell surface by proteolytic cleavage of the core protein (1). The site of cleavage for syndecan-1 has been localized to within 9 amino acids adjacent to the extracellular face of the plasma membrane (44). Although the identity of the syndecan cleaving enzyme is unknown, its activity is cell surface-associated and can be inhibited by peptide hydroxamates and tissue inhibitor of metalloproteinase-3 (TIMP-3) but not by TIMP-1 or -2 or by inhibitors of aspartic acid, cysteine, and serine proteases (44). These properties suggest that the enzyme is a cell surface zinc metalloproteinase that belongs to the TIMP-3-sensitive ADAM (a disintegrin and metalloproteinase) family. Interestingly, this mechanism appears to be responsible for the shedding of all four mammalian syndecans despite the lack of sequence similarity in the juxtamembrane region, implicating conservation of cleavage site secondary structures or the contribution by other regions of the proteoglycans for substrate recognition by the shedding enzyme. The glypicans can be shed from the cell surface (at least from cells in culture) possibly by shed syndecan-1 ectodomains has also been observed with carcinoma cell lines (49).

Shedding can also potentially regulate receptor signaling events by rapidly reducing the amount of cell surface HS. For example, shedding of syndecan-1 ectodomains can also function as potent inhibitors of heparin-mediated FGF-2 mitogenicity via the NA domains of their HS chains (48). However, when the same syndecan-1 ectodomains are treated with platelet heparinase, as would happen during tissue injury, the enzyme digests the NA domains. This releases the enzyme-resistant NS domains that now activate FGF-2 mitogenicity (48). Indeed, mitogenically active concentrations of the heparin-like NS domains are found in acute wound fluids. Thus, the macro-organization of HS chains has a functional significance. Inhibition of cell proliferation by shed syndecan-1 ectodomains has also been observed with carcinoma cell lines (49).

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Evolutionary and Future Perspectives

The syndecans apparently arose during the major metazoan radiation of the Cambrian period because their core protein genes are similarly organized and present in conserved forms in nematodes, arthropods, and chordates. Metazoans evolved, in large part, because of their ability to establish epithelia that generate an
intercellular environment segregated from the outside world. Syn-
decans are required to maintain such epithelia. Once an extracel-
lar space was segregated, which emerged with gastrulation and the appearance of HS in Cnidaria, the evolution of extracellular signaling and matrix proteins was possible. Thus, HS-protein inter-
actions seem to be based on an evolutionarily conserved extra-
cellular space. This idea is consistent with (i) the remarkable preval-
ence of such proteins that depend for their function on binding HS, (ii) the many distinct protein structures responsible for this binding, (iii) the involvement of most of these proteins in processes that are fundamental to an organism’s survival, and (iv) the apparent lack of major evolutionary changes in HS-protein interac-
tions. Like the RNA code, this HS code is degenerate, redundant, and sequence-specific; its fidelity is based on interactions with proteins and its significance is in directing cellular behavior. How-
ever, unlike the RNA code, this code is not based on a direct template mechanism, is restricted to metazoans, and appears to depend on cellular differentiation.

Despite the seemingly indiscriminant binding properties of HS chains, as we learn more about specific HSPG–protein interactions, HS is proving to be physiologically significant. For example, the initial HS chain-modifying enzyme, NDST-1, is the most widely distributed NDST iso-
form involved in HS biosynthesis, yet mice made null for this enzyme show incomplete maturation of type II pneumocytes, a strikingly limited defect (50, 51). Another example is the absence of kidney development in mice with a disrupted HS-2-O-sulfotrans-
ferase gene (52). Human genetic diseases are also revealing unex-
pected specific functions for HSPGs. For example, the formation of benign bone tumors (hereditary multiple exostoses) is a consequence of mutations in HS copolymerases (53), and limitations on pre- and postnatal somatic growth are imposed by glypic-an-3 (Simpson-Golabi-Behmel syndrome) (54). Technologies recently in-
troduced for the sequencing of HS chains (55) and for the chemical synthesis of specific HS sequences (56) will be available in the near future to help elucidate the molecular mechanisms underlying such distinct developmental and physiological processes.

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