Heparan Sulfate: A Complex Polymer Charged with Biological Activity

John M. Whitelock*,† and Renato V. Iozzo*,‡

Graduate School of Biomedical Engineering, University of New South Wales, Kensington, Sydney, New South Wales 2052, Australia, and Department of Pathology, Anatomy and Cell Biology and the Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Received March 4, 2005

Contents

1. Introduction

Heparan sulfate (HS) is a complex and highly active biopolymer that is synthesized as an alternating copolymer of hexuronic acid and glucosamine and modified at various positions with sulfate, which affects and controls its biological activities.[1,2] Heparin, which we consider as an oversulfated intracellular variant of the ubiquitous HS, was first discovered in 1916 as an inhibitor of coagulation and was further developed for the next 20 years when it was first tested in patients as an anticoagulant drug in the mid 1930s.[3] Indeed, heparin is second only to insulin as what could be thought of as a very successful natural therapeutic agent. It received its name, "hepa"rin/"hepa"ran, because hepatic tissue was a common and abundant source from which it was first isolated and studied. HS was originally discovered as an impurity of heparin preparations and named heparitin sulfate.[4] These names remain in place today and tend to cause some confusion because it is becoming clear that both heparin and heparan sulfate are very closely related and may share many structural and functional activities. The mechanism of action of HS includes specific, noncovalent interaction with various proteins, a process that affects the ultimate fate of the protein, that is, topographical destination, half-life, and bioactivity. The HS roles as co-receptors for various receptor tyrosine kinases and its ability to affect morphogen gradients, and thus development and organogenesis, are important attributes that have raised considerable attention in the past decade.

This review focuses on the synthesis and roles of HS in both biology and pathology. It also describes the interactions with various groups of proteins including cytokines and growth factors, which have been shown to be important in controlling the biological activities of the bound factor. It concludes by reviewing the ways in which the structure of HS has been analyzed biochemically and investigated for its possible roles in diseases and pathologies affecting humans.

2. Biosynthesis

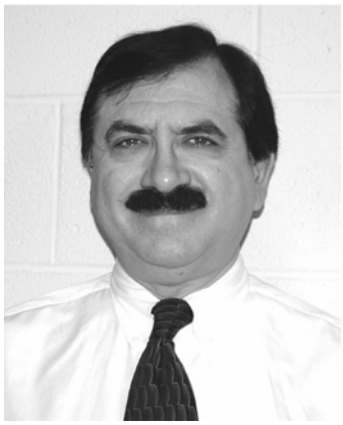
HS biosynthesis is a complex multistep process that occurs in a very specific and sequential manner via membrane-bound enzymes in the endoplasmic reticulum and the Golgi apparatus. These enzymes utilize various monosaccharide building blocks after they have been activated to their UDP forms and transported to the endoplasmic reticulum where they are added to the growing HS chain. They are transported across the membrane of the Golgi by specific transporters, which are also important for the exit of the UMP and inorganic phosphate byproducts of the various chemical reactions.[5,6] Sulfate is also activated and transported to the endoplasmic reticulum and Golgi by inclusion of free sulfate into 3′-phosphoadenosine 5′-phosphosulfate (PAPS), but the molecules involved in its transmembrane transport are less understood. The various enzymes involved in the biosynthesis of HS, together with a very thorough summary of their substrate specificities, their genetic nomenclature, and the characteristics of cell lines where their genes have been mutated, are elegantly described in a recent review by Esko and Selleck.[7]

As potential HS proteoglycans transit the Golgi, specific amino acid sequences are identified that target each of the protein cores to be modified with HS. In mammalian systems, there are at least 13 full-time HS proteoglycans and several part-time ones. Specifically, there are four transmembrane syndecan HS proteoglycans, six glycosylphosphatidylinositol (GPI)-anchored glypicans, and three basement mem-

* Corresponding authors. E-mail addresses: j.whitelock@unsw.edu.au; iozzo@mail.jci.tju.edu.
† University of New South Wales.
‡ Thomas Jefferson University.

10.1021/cr010213m CCC: $53.50
Published on Web 06/25/2005

John Whitelock received a Ph.D. degree in 1990 from the University of Technology, Sydney, studying the expression and secretion of metalloproteinases and their inhibitors in breast cancer cells. He completed postdoctoral training in the U.S.A., at the University of Alabama in Birmingham, Alabama, and returned to Australia in 1992 to take up a role as a research scientist with the Cooperative Research Center for Cardiac Technology in laboratories of the Commonwealth Scientific Industrial Research Organization. Dr. Whitelock is currently an Associate Professor within the Graduate School of Biomedical Engineering at the University of New South Wales in Sydney, Australia. Here he manages a research group investigating the role of proteoglycans in tissue engineering and their application in the development of diagnostic biomarkers for disease along with their potential use in novel biomaterials. He also has fundamental interests in the biochemistry of proteoglycans, particularly focused on the structure of heparan sulfate and the aspects of its structure that control biological activity. His major contribution to this field has been an examination of the heparan sulfate protoeglycan perlecan, a component of most connective tissues and basement membranes, and he has shown that it can possess different biological activities depending upon cell source.



Renato Iozzo is currently a Professor of Pathology and Cell Biology and Director of the Extracellular Matrix Program at the Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA. After receiving an M.D. degree summa cum laude from the University of Florence, Italy, he moved to the Department of Pathology at the University of Washington, where he completed a five-year Residency/Fellowship. Following a six-year faculty appointment at the University of Pennsylvania, he moved to Thomas Jefferson University. Dr. Iozzo has received many awards including the Benjamin Castleman Award from the International Academy of Pathology, the Junior Faculty Research Award from the American Cancer Society, the Burlington Resources Foundation Faculty Achievement Award, and the Faculty Research Award from the American Cancer Society. In 1989, he was elected to the Pluto Society, the American Association of Academic Pathologists. He was the chair of the Gordon Research Conference on Proteoglycans in 2000, and a council member of both ISMB (2001–2004) and ASMB (2001–2003). He is currently the President Elect of the American Society for Matrix Biology. Dr. Iozzo is on the editorial board of many journals, including *Laboratory Investigation* (fourth term), the *Journal of Biological Chemistry* (second term), and the *Journal of Clinical Investigation* (third term), and is currently Review Editor for *Matrix Biology*. His research focuses on the biology of proteoglycans and their roles in matrix assembly, cell growth, and angiogenesis. He has published over 200 peer-reviewed articles and numerous reviews and edited two books on proteoglycans.

brane HS proteolgycans, including collagen XVIII, agrin, and perlecan[8] (Figure 1A).

Clearly, the genetic information that regulates the attachment of HS is encoded in the protein core, insofar as most eukaryotic cells synthesize both HS and chondroitin sulfate (CS). Surprisingly, cells that are capable of manufacturing both HS and CS, place the correct glycosaminoglycan with a high degree of fidelity. For instance, all the full-time HS proteoglycans shown in Figure 1 are almost exclusively substituted with HS chains, at least when they are extracted from tissues or naturally synthesized in vitro by various cells. In contrast, members of the aggrecan and small leucine-rich proteoglycan families are almost exclusively substituted with CS or dermatan sulfate (DS) chains.

As mentioned above, most HS proteoglycans contain repetitive serine−glycine amino acid sequences flanked by clusters of acidic amino acid residues, primarily aspartic acid and glutamic acid (Figure 1B). These acidic boxes are quite variable in size and sequence, and their presence enhances the chance of a protein to be glycanated, that is, substituted with at least one glycosaminoglycan side chain.[9,10] Esko and co-workers fused short segments (20−30 amino acid residues) of various proteoglycan protein cores to the Ig-binding domain of protein A. The secreted chimeric proteins were then used as a tool to measure the subsequent substitution with either HS or CS. Using this clever approach, these authors provided a first glimpse at the specificity of glycanation: particular protein sequences favored the attachment of CS, while others favored the attachment of HS. Moreover, mutations of key acidic residues within this region can give more CS than HS.[9,10] Studies involving perlecan have identified an acidic sequence located upstream of the glycosaminoglycan attachment sites that is an important factor in the addition of HS.[11] Other investigators have shown that domains either C-terminal to the HS attachment region[12,13] or even fused to totally unrelated proteins[14] have a major effect on dictating the degree of HS that is attached to the serine residues in the N-terminal domain of perlecan. Similar distal sequences in the protein core of glypican-1 have a major effect on the attachment of HS to its protein core.[15] Collagen XVIII derived from various tissues is almost exclusively a HS proteoglycan, whereas that purified from human kidney embryonic cells as a recombinant proteoglycan is a hybrid species containing both HS and CS sugar moieties.[16] These studies highlight the difficulty in predicting the site of HS attachment based solely on a specific amino acid sequence. In addition, tissue- and cell-specific controls of glycanation play a significant role. Deciphering the exact mechanisms that control the attachment of HS to distinct protein cores will be a challenging endeavor of future research.

Both CS and HS proteoglycans have an identical sequence in the initial saccharides that are added to the serine on the protein core. This sequence is

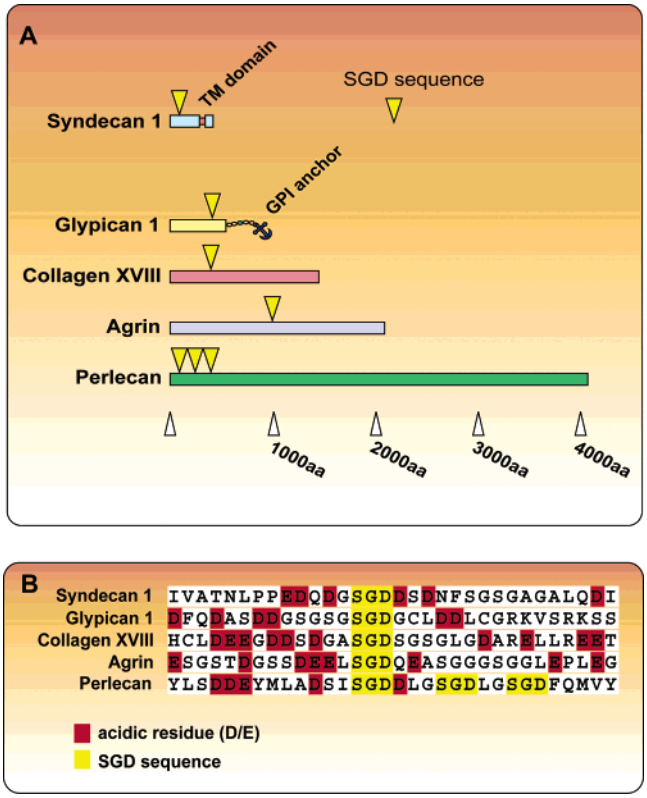


Figure 1. Schematic representation of the known full-time human HS proteoglycans (A) and their amino acid sequences around the predominant HS-binding site, SGD (B). While collagen XVIII, agrin, and perlecan are encoded by single genes, there are four syndecan and six glypican genes in the human genome. The various protein cores are in scale in terms of amino acid number. Depicted are only the SGD sequences, which, almost exclusively, dictate the covalent substitution of the protein core with HS chains. However, each protein core depicted in panel A possesses other potential HS attachment sites, and in some instances, hybrid proteoglycans containing both HS and CS chains have been reported. Note also that the sequence of human collagen XVIII has not been formally shown to be substituted with HS chains.

known as the "linkage tetrasaccharide" and comprises β-xylose, β1−4-galactose, β1−3-galactose, and β1−3-glucuronic acid. The first reaction, catalyzed by the enzyme UDP-xylosyl transferase, results in the addition of a xylose in the β-anomeric configuration to the serine of the triplet sequence (SGD) via an O-linkage between the serine and the C1 of the xylose (Figure 2A). The next reaction results in the addition of a galactose via a β1−4 linkage to the xylose by UDP-galactosyl (UDP-Gal) transferase I. This is followed by the addition of another galactose; however, due to the growing complexity of the chain and its effect on enzyme−substrate specificity, this reaction is catalyzed by a different enzyme, UDP-Gal transferase II. This enzyme also utilizes UDP-galactose but results in the galactose being linked via a β1−3 linkage. The final monosaccharide of the linkage tetrasaccharide is added by UDP-glucuronic acid (GlcA) transferase I, which adds a UDP-glucuronic acid to the galactose via a β1−3 linkage.

It is at this step in the synthesis where HS assembly diverges from that of CS. In the case of CS, an enzyme known as *N*-acetylgalactosaminyl transferase adds *N*-acetylgalactose (GalNAc) to the terminal GlcA residue of the linkage tetrasaccharide.[17] The HS synthesis arm, instead, utilizes *N*-acetylglucosaminyl (GlcNAc) transferase I (also known as *extl*2), which adds a *N*-acetylglucosamine (GlcNAc) to the GlcA at the nonreducing end of the linkage tetrasaccharide via an α1−4 linkage. There is some evidence that this activity resides within two separate enzymes and that they may also catalyze the addition of GalNAc to the linkage tetrasaccharide via an α1−4 linkage.[18] In contrast, the biosynthesis of CS requires the addition of a GalNAc in the β1−4 form. Notably, a sulfate present at the 4-position of the preceding galactose (Gal) residues[18,19] affects the activity of the GalNAc transferase, which results in prevention of further chain polymerization. Phosphorylation of some of these residues has also been suggested to play a role in modulating the activity of the transferase.[20]

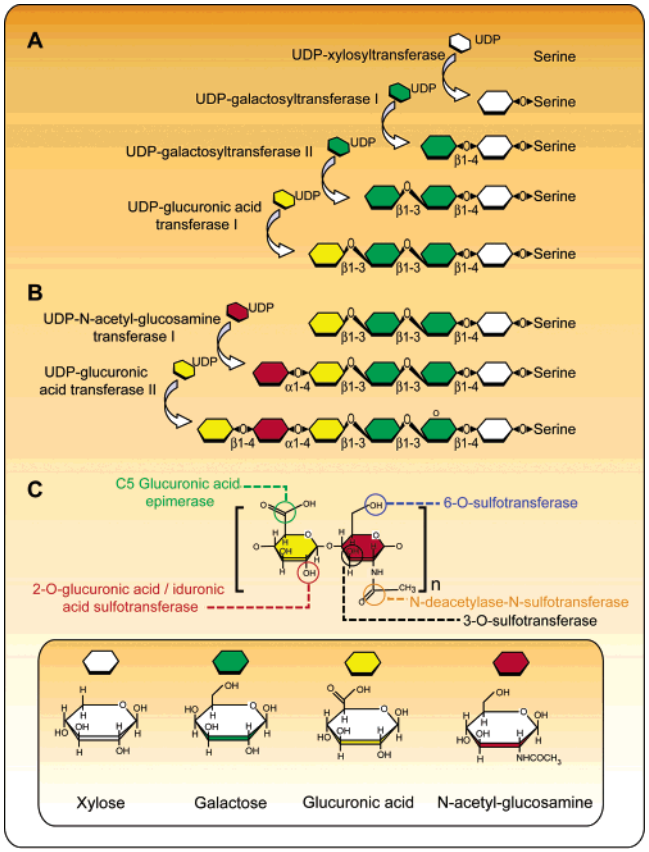


Figure 2. Schematic representation of the complex biosynthetic steps leading to the construction of a mature HS chain. (A) Chain initiation: the biosynthesis of the linkage tetrasaccharide region of HS is shown. This sequence is also utilized for attachment of CS and DS glycosaminoglycans to other protein cores. The curved arrows indicate the position at which each modification takes place. The structure of each monosaccharide and their color-coded symbols are shown in the bottom panel. (B) Chain elongation: the alternating copolymeric nature of HS is produced by the action of an enzyme complex of both *ext1* and *ext2* gene products, which confer the UDP-*N*-acetylglucosamine transferase I and UDP-glucuronic acid transferase II activity, respectively. (C) Chain modification: the repeating disaccharide unit of HS is further modified by various enzymes as depicted. For additional information, see the text.

The elongation of HS chains, that is, the consecutive addition of alternating GlcA and GlcNAc residues, is mediated by the action of two main polymerization enzymes, GlcNAc transferase II, which adds a GlcNAc residue via an α1−4 linkage, and GlcA transferase II, which adds the GlcA residue via a β1−4 linkage (Figure 2B). These enzymes are the products of the *ext1* and *ext2* tumor suppressor genes, respectively, and are part of a large hetero-oligomeric complex that is membrane-bound in the Golgi apparatus. The exact roles of each of the gene products and their relationship to each of the polymerization reactions is very complex, and it seems that both need to be present in the complex for successful chain polymerization to take place.[21] Results from mutational analysis of sequences within the *ext1* gene suggest that this domain is responsible for the GlcA transferase activity.[22] Recently, a third member of the *ext* family of genes, having homology to the *extl3* gene in humans,[23] was identified in *Drosophila* and shown to have both GlcNAc transferase I and GlcNAc transferase II activity,[24] suggesting that it may play a pivotal role in the synthesis of HS.

As the HS chain is extended, it can be further modified at various positions in different ways (Figure 2C). This process is not merely random and occurs in a very specific and orderly manner with the modifications achieved at each step affecting the substrate of the enzyme that performs the next modification.[25] Some of the GlcNAc groups along the HS chain can be N-deacetylated and then N-sulfated, a process that is catalyzed by the enzyme GlcNAc N-deacetylase/N-sulfotransferase. The modification of the HS chain at this position is thought to occur as a result of tissue-specific expression of the four different isoforms of this enzyme.[26] This is particularly pertinent in the case of the synthesis of heparin, which has a greater proportion of modified regions leading to a greater proportion of N-sulfation, glucuronic acid epimerization, and sulfation of the resultant iduronic acid residue. It has been shown that cells that do not produce the classical heparin sequences also express the same enzyme that is responsible for the synthesis of heparin in mastocytoma cells.[27] The role of these enzymes will be discussed later in more detail in relation to the results obtained from knockout studies. Molecular modeling and studies using fusion proteins have indicated that the catalytic domain of these enzymes is in the C-terminal half of the enzyme.[28] It has also been shown that factors other than the expression of enzyme isoforms, including the presence of the correct UDP-monosaccharides in the lumen of the Golgi, can markedly affect HS biosynthesis.[29]

A further modification of the HS chain, which tends to occur after N-sulfation, is the epimerization of glucuronic acid into iduronic acid by the action of the enzyme C5-GlcA epimerase (Figure 2C). This enzyme converts the carboxyl group on the C5 position of a glucuronic acid so that it is oriented below the hexose ring. Interestingly, this enzyme cannot convert glucuronic acid residues that are surrounded by iduronic acid residues already sulfated at C2 or by glucosamine residues that have been O-sulfated.[30] These findings support the hypothesis that this enzyme acts prior to some of the other modifications seen in HS chains.[31] It also supports the idea that the substrate specificity of each of the enzymes is modulated by the action of enzymes acting later in the HS biosynthetic cascade. The addition of sulfate groups at C2 of either iduronic or glucuronic acid is catalyzed by the enzyme 2-O-glucuronic acid/iduronic acid sulfotransferase.[32] The action of the sulfotransferase affects the activity of the epimerase. Moreover, their targeting to the endoplasmic reticulum and Golgi is dependent upon the presence of both enzymes. C5 epimerase activity reduced in cells lacking the 2-O-sulfotransferase is restored when the cells are transfected with the 2-O-sulfotransferase gene. This suggests that these two enzymes may form functional complexes in the Golgi and that this colocalization relates to the rapid rate of HS synthesis in certain cells.[33] Further support of this interconnectivity between the C5 epimerase and the 2-O-glucuronic acid/iduronic acid sulfotransferase is provided by the finding that the latter enzyme had an affinity for iduronic acid-containing disaccharides about 5-fold greater than that for glucuronic acid-containing disaccharides.[34] A related enzyme that catalyzes the addition of sulfate to C2 of either iduronic or glucuronic acid in CS/DS chains has been cloned.[35]

Further sulfation of the HS chain can occur at the C6 and C3 positions of glucosamine residues catalyzed by the enzymes 6-O-sulfotransferase and 3-O-sulfotransferase, respectively (Figure 2C). Both enzymes, like the N-sulfotransferases, exist as multiple isoforms.[36−38] Interestingly, the 6-O-sulfotransferase that catalyzes the modification in HS is a member of a larger family of enzymes that catalyzes the transfer of sulfate from PAPS to galactose, galactosamine, and glucosamine. So far there have been six members of this family identified,[37] including those that perform some of the relatively common modifications identified in CS.[38]

As further evidence of the inherent linked activities of the various enzymes, the 6-O-sulfotransferase enzyme acts in concert with the 3-O-sulfotransferase to create the specific binding site for the antithrombin III protein.[39] The level of sulfation at the C2 position of glucuronic and iduronic acids is important in determining the level of activity of 6-O-sulfotransferase 3 insofar as the enzyme has a preference for sequences with higher amounts of 2-sulfation.[40] The last family of enzymes that are involved in the modification of HS are the 3-O-sulfotranserases, which add a sulfate group to C3 of already sulfated glucosamine residues.[41] This modification is more common in HS attached to serglycin produced by mast cells than the HS produced by other cell types. However, its activity in endothelial cells to produce a noncoagulant surface is critical to the normal functioning of these cells in the vasculature. The activity of a 3-O-sulfotransferase was first described in 1988 when it was shown that it was the last step in the production of the anti-thrombin binding region, which necessitated the enzyme adding a sulfate to C3 of an N-sulfated, 6-sulfated glucosamine.[42] The production of anticoagulant HS

by endothelial cells is thought to be controlled in a rate-limiting fashion by the 3-O-sulfotransferase 1 isoform,[43] whereas the production of anti-thrombin III binding sites in HS can also be achieved by other isoforms, such as 3-O-sulfotransferase 5[44] but not by either the 3-O-sulfotransferase 2 or 3A isoform.[45] Recently, the 3-O-sulfotransferase 5 isoform has been shown to be highly expressed in neural tissue where it was important in the generation of tetrasulfated disaccharide sequences.[46] This isoform has a broader substrate specificity than either 3-O-sulfotransferase 1 or 3-O-sulfotransferase 3 and is capable of adding sulfate to both N-sulfated glucosamine and glucosamine residues with free amino groups.[47] In contrast, the 3-O-sulfotransferase enzyme can transfer sulfate onto glucosamine residues with free amino groups.[48] The way in which these enzymes bind and interact with HS controls their activity and substrate specificity. Studies using 3-O-sulfotransferase 1 have shown that a group of lysine residues near the C-terminus becomes more exposed on the surface of the enzyme suggesting that binding to HS causes a conformational change. Constructs that lacked the C-terminus were shown to be much less active than full-length enzyme.[49] However, results from the same laboratory have indicated that other basic residues throughout the length of the enzyme are also important for enzyme−HS binding and activity.[50] The activity of 3-O-sulfotransferase 1 was shown to be independent of the level of 2-sulfation occurring in a HS chain, except when the glucosamine had an unsulfated iduronic acid residue on its nonreducing side.[51]

Another important realization is that HS proteoglycans at the cell surface or in basement membranes are in a dynamic state: they are constantly internalized and degraded and partially recycled to the cell surface with various $t_{1/2}$ in the range 4−24 hours[52,53] with the vast majority being turned over by 48 hours.[54] HS is not only subjected to classical intracellular lysosomal degradation,[55] a process modulated by the positions of the sulfate groups,[56] but it is also processed by extracellular sulfatases.[57] These enzymes exert their activity on cell surface and extracellular HS proteoglycans that modulate *Wnt* signaling in embryonic development.[58,59] One such enzyme, *Qsulf1*, has been studied in more detail and shown to specifically remove the 6-sulfate groups from sulfated glucosamine residues within an HS chain,[57] thereby affecting growth factor signaling such as bone morphogenetic protein (BMP)[60] and fibroblast growth factor 2 (FGF2).[61] This contributes further to HS structural and functional complexity as a biopolymer.

3. Genetic Defects in Heparan Sulfate-Modifying Enzymes: Unexpected Phenotypes

There are various ways to study the importance of HS in development and the role it plays in the control of cell and tissue behavior. One way is to interfere with the biosynthetic pathway described in detail above by deleting or inactivating different enzymes. Interfering with enzymes earlier in the pathway results in a greater reduction in the final amount of HS produced. Inhibiting the enzymes involved in the generation of the linkage tetrasaccharide will affect all CS, DS, and HS glycosaminoglycan synthesis. A second way is to interfere with the production of the protein component of a specific HS-containing proteoglycan, such as the core protein of perlecan, collagen XVIII, agrin, syndecan 1−4, or glypican 1−6 by knocking out specific gene sequences. Results from these experiments will not be discussed in detail as part of this review, but only a few selected examples will be covered below.

Mutations in some of the enzymes involved in the synthesis of the linkage tetrasaccharide region have been studied in *Caenorhabditis elegans* where the genes *sqv-3, sqv-7,* and *sqv-8* have been shown to be involved in the synthesis of proteoglycans.[62,63] *Sqv-8* has homology to the glucuronyl transferase I enzyme that links glucuronic acid to the disaccharide galactose β1−4 *N*-acetylglucosamine present in the linkage region, whereas *sqv-3* has homology to the galactosyl transferase enzyme that links galactose to the *N*-acetylglucosamine. Mutations in these genes severely affect proteoglycan production resulting in an inhibition of epithelial invagination, which is a critical event in the formation of tubular structures and the process of organogenesis in multicellular organisms.[62,63] Mutations in the *sugarless (sgl)* gene in *Drosophila*, which encodes the homologue of UDP-glucose dehydrogenase that converts glucose to glucuronic acid, has also been shown to severely inhibit the production of proteoglycans resulting in profound effects on the development of progeny.[64]

Significant evidence is accumulating on the role of HS proteoglycans in determining the shape of morphogen gradients and morphogen activities by contributing to the spreading of morphogenetic cytokines from their cellular sources toward the responding cells.[65] For example, Dally like protein (*Dlp*) in *Drosophila* encodes for a protein with homology to mammalian glypicans and has been shown to be critical for signaling of the morphogen, wingless (*Wg*).[66,67] Specifically, Selleck and co-workers have elegantly shown that, contrary to predictions from overexpression studies, *dlp* is required to control *Wg* levels in the matrix[66] since *dlp* mutants show ectopic activation of *Wg* signaling at the presumptive wing margin and cause a local increase in *Wg* levels (Figure 3). Moreover, *dlp* somatic cell clones disrupt the gradient of extracellular *Wg*. The activity of *dlp* appears to be regulated by the secreted protein Notum—an enzyme that shares homology with a plant hydrolase thereby limiting *Wg* signaling[66] and that causes the shedding of the *dlp* into the extracellular milieu by clipping the proteoglycans at the cell surface close to its GPI anchor.[68] Thus, enzymatic modification of HS proteoglycans by a secreted hydrolase can affect morphogen levels in the matrix. This is an additional and novel mechanism by which morphogen gradients are controlled by HS proteoglycans.

Glypican-3 knockout mice have been shown to have defects in the growth-factor signaling molecule, *Wnt*.[69] Filmus and co-workers have recently provided convincing genetic and molecular evidence indicating

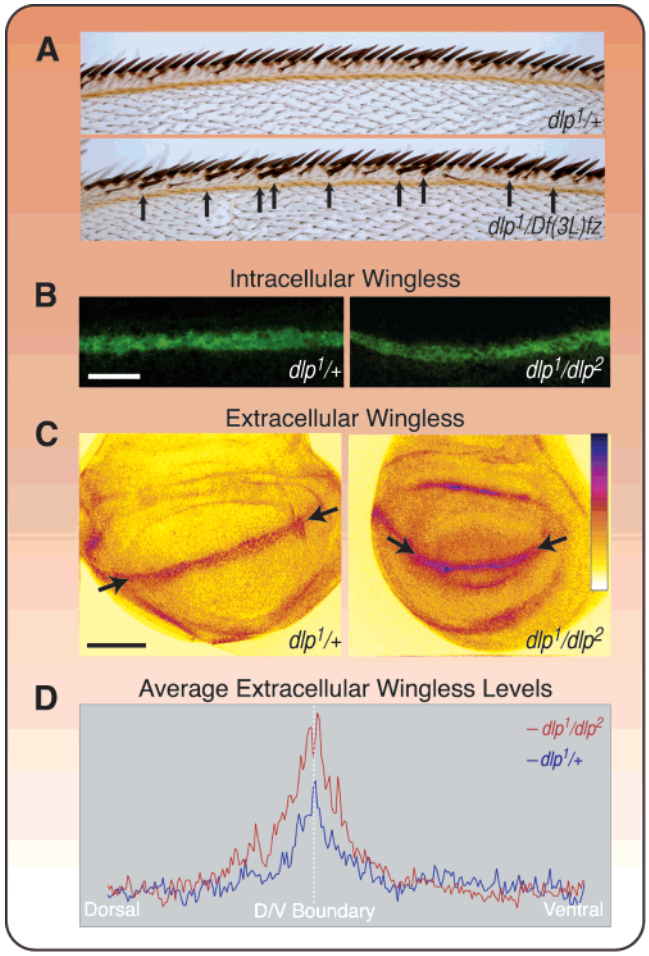


Figure 3. Loss of a *Drosophila* glypican (dally like, *dlp*) produces elevated wingless (*Wg*) signaling and alters the distribution of extracellular *Wg*. (A) *dlp* mutant wings show ectopic mechano-sensory bristles, indicative of increased levels of *Wg* signaling. A dorsal view of the anterior edge of a heterozygous control (dlp^1/+) is compared with *dlp*/*dlp* mutant adult wings. Stout mechano-sensory bristles are found along the edge of the normal wing; more widely spaced, thinner chemosensory bristles form another row just posterior to the first. (B) *Wg* is produced by a stripe of 3−4 cells along the dorsal−ventral boundary of the developing wing disk, and *Wg* expression is unchanged in *dlp* mutants. Portions of heterozygous and *dlp* mutant wing disks stained for intracellular *Wg* protein are shown; anterior is to the left and ventral is down. *dlp* mutant wing disks show comparable levels of intracellular *Wg* compared with heterozygous controls. Scale bar = 50 μm. (C) Heterozygous and *dlp* mutant wing disks stained for extracellular *Wg* protein; anterior is to the left and ventral is down. *Wg* expressed at the dorsal/ventral boundary (marked by arrows) diffuses across the tissue to form a concentration gradient. *dlp* mutant wing disks show elevated levels of extracellular *Wg* compared with heterozygous controls. Pseudocolor representations of the confocal images are shown to permit easy visualization of differences in *Wg* immunodetection (lookup table is inset). Scale bar = 50 μm. (D) *dlp* mutant wing disks show locally elevated levels of extracellular *Wg*. The plots show the average intensity of extracellular *Wg* staining along the dorsal/ventral axis of the wing pouch from six dlp^1/+ and six dlp^1/dlp^2 disks. Note that *Wg* levels are elevated in the central half of *dlp* mutant wing disks compared to controls. At the periphery of the disk, extracellular *Wg* levels in *dlp* mutants are no different from controls.

that loss of glypican-3 leads to inhibition of the noncanonical *Wnt/c-jun* N-terminal kinase (JNK) signaling pathway, while concurrently causing an activation of the canonical *Wnt/β*-catenin signaling pathway.[69] Interestingly, ectopic expression of glypican-3 in mesothelioma cells and the concurrent activation of JNK signaling is associated with an enhanced response to *Wnt5a*,[69] a gene that is upregulated in a number of human malignancies[70] and has been mapped to chromosome 3p14-p21, a chromosomal region often linked to gene aberrations associated with various malignancies.[71] Glypican-4 has been shown to be important in the neural patterning of the forebrain in *Xenopus* development, where it is hypothesised to bind to FGF2 and prevent the apoptosis of the primordial cells residing in the developing tissue.[72] Syndecan-3 plays an important role in the generation of cartilage tissue and the maintenance of the growth plate and correct limb organogenesis by interacting with the Indian hedge hog (*Ihh*) growth factor.[73] These findings underscore the importance of HS as the common denominator in these proteoglycans, although glypicans and syndecans have been shown to have separate and distinct roles in the development of neural and visual tissue in *Drosophila*.[74]

There is building evidence that the HS and its sulfation pattern contain the code for the successful generation of various types of tissue[75] including neural tissue.[76] Other experimental findings that support the idea that HS is important in the establishment and maintenance of morphogen gradients have emerged from studies performed in organisms that totally lack HS as a result of genetic ablation of the HS polymerizing enzymes, *ext1* and *ext2*. Studies in *Drosophila* identifying mutations in either the *ext1* homologue, *tout velu (ttv)*, or *ext2* homologue, *sister of tout velu* (*sottv*), have demonstrated that HS is important in establishing morphogen gradients in the developing embryonic tissue and that disrupting its synthesis disrupts three major signaling pathways, namely, *hedgehog* (*Hh*),[77−80] *Wg*,[80] and *decapentaplegic (Dpp)*[80] pathways. Lack of HS polymerase causes not only a marked reduction in HS content in the mutant larvae but also a decrease in hedgehog protein levels, suggesting that the reduced range of hedgehog protein in the absence of HS may cause an increased lability of the morphogen.

Similar phenotypes have been noted in mice lacking the *ext1* gene, where the embryos fail to develop early in gestation due to a catastrophic failure in gastrulation.[81] *Ext-1*-null mice produce no HS and possess an abnormal distribution of the morphogen Indian hedgehog (Ihh) that fails to associate with the cell surface.[81] To define the developmental role of HS in mammalian brain, Yamaguchi and co-workers have conditionally disrupted the HS-polymerizing enzyme *ext1* in the embryonic mouse brain.[82] Notably, these mutant animals exhibited a complex phenotype with patterning defects composed of those caused by numerous HS-binding morphogens. Specifically, the mutant brains showed severe guidance errors in the major commissural tracts, thereby revealing that HS is crucial for mammalian brain development.[82] Mice carrying a hypomorphic mutation in *ext1* exhibit an increased range of Ihh signaling due to the lack of

HS, which fails to initiate a suitable concentration gradient of the Ihh.[83] Notably, mutations in the zebrafish *ext* orthologs, *dackel* and *boxer*, are responsible for missorting of retinal ganglion cells in the optic tract.[84] It is also interesting to note that in zebra fish, three different isoforms of *ext1* have been identified. These isoforms have unique tissue-specific distributions and respond to the regulatory signals provided by sonic hedgehog *(Shh)* in a differential manner.[85] In humans, mutations in the *ext* genes give rise to a dominantly inherited syndrome that gave the genes their names, multiple exostoses.[86] The affected individuals have abnormalities in bone growth that result in bone dysplasias typified by the presence of bony outgrowths that have been hypothesized to be due to the incorrect signaling of *Ihh*, which controls the rate of chondrocyte differentiation.[87]

The sulfateless *(sfl)* gene in *Drosophila*, which encodes the homologue of the N-deacetylase/ N-sulfotransferase enzyme, plays an important role in *Wg* signaling.[88] Mutations in this gene result in aberrant migration of mesodermal cells due to disruption of heartless and breathless (homologues of the fibroblast growth factor receptor genes of mammals) signaling.[89] In mice, gene-targeting of the N-deacetylase/N-sulfotransferase-1 (NDST-1) enzyme results in some embryonic lethality, whereas the greater proportion of embryos survive to birth but die soon after from pulmonary insufficiency and cyanosis due to malformed lungs.[90] These mice exhibited a widespread reduction of N-sulfate content in the HS isolated from various tissues; this is contrasted by the specific decrease of N-sulfation of HS present in mast cell granules in those mice that were homozygous for a gene mutation in the NDST-2 gene. Furthermore, when HS was isolated from various tissues such as kidney, lung, liver, and bladder from the NDST-2 defective mice, the overall structure of the HS was similar to that isolated from the same tissues from wild-type animals.[91] In contrast, the HS isolated from the NDST-1 deficient mice has a lower overall sulfation resulting from a concomitant decrease in the activity of the O-sulfotransferases, supporting the hypothesis that N-sulfation carried out by NDST-1 is a key and early step in HS biosynthesis that controls the activity of the subsequent O-sulfotransferase enzymes.[92]

Mice defective in the NDST-2 activity, apart from having effects in the HS in their mast cells, which affected the localization and concentration of both histamine and some of the mast cell proteases, develop to birth and through maturity normally. These results suggest that the expression of the enzyme is not critical for early embryonic development but is critical for the production and storage of histamine and proteases in mast cell granules.[93,94] Disruption of the NDST-2 gene prevents the generation of fully sulfated heparin, which in turn causes abnormal packing of mast cell proteases. Thus, heparin is essential for the storage and biological activity of specific mast cell granule proteases. This affects an important step in immune surveillance and anti-infectious activity. Interestingly, when melanomas were grown in these mice, they grew to a significantly larger size and contained more thrombosis than those tumors grown in control mice.[95] This suggests that the enzyme may be important in the suppression of tumor growth by some as yet undefined role that may either involve the heparin directly or the proteases that are stored within the mast cell granules.[95]

In *C. elegans*, mutations in either the C5 epimerase, 2-O-sulfotransferase, or 6-O-sulfotransferase have been studied.[96] Each animal that had a reduced expression in a single enzyme exhibited abnormalities in cell signaling pathways involved in neuronal guidance (*sax3/Robo; kal-1/Anosmin-1*). Animals with mutations in two of the enzymes exhibited a greater reduction in signaling.[96] Interestingly, the phenotype was worse when the 6-O-sulfotransferase-null animals were crossed with either the C5 epimerase- or 2-O-sulfotransferase-null animals suggesting that the presence of the sulfate on the 6 position on some of the glucosamine residues might increase the efficiency of binding between the morphogens and HS sequences harboring 2-O sulfate modified iduronic acid/glucuronic acid residues. The importance of the 2-O-sulfotransferase in *C. elegans* development has been recently confirmed insofar as cells from the mutant animals exhibit abnormal migratory activity.[97]

The homologue of the 2-O-sulfotransferase gene in *Drosophila* is known as *pipe*.[98] This gene has arisen from exon duplication[99] and is involved in embryonic patterning, a process that is critical in the determination of both dorsal and ventral surfaces of the developing progeny.

Mice deficient in 2-O-sulfotransferase activity survive to birth but die soon after because 2-O-sulfation is absolutely necessary for renal development, where it is involved in the growth and branching of mesenchymal progenitor cells.[100] The HS isolated from embryonic fibroblasts from these mutant animals shows a compensatory increase in N- and 6-sulfation resulting in HS that could signal fibroblast growth factor receptors as effectively as native HS.[101,102]

The 6-O-sulfotransferase enzymes are important in the generation of limbs in the developing chicken embryo. It is interesting to note that isoenzymes in this tissue have very distinct localization patterns with the 6-O-sulfotransferase 1 expressed more highly in the anterior region of the developing limb and the 6-O-sulfotransferase 2 isoform expressed at greater levels in the posterior region.[103] This suggests that these enzymes have a very important role in generating the correct distribution and gradients of morphogens by providing specific regions within HS with the correct sulfation sequences (signature or cassette) that allow the precise binding and activation of the morphogens. Throught use of morpholino antisense oligonucleotides to inhibit the transcription of both the 6-O-sulfotransferase 1 and 2 genes, it was found that these enzymes have an important role in the development of zebrafish muscle.[104] In humans, mutations in the 6-O-sulfotransferase gene on chromosome 16 cause macular corneal dystrophy.[105] Although the exact molecular mechanism for this ocular abnormality is not understood, it is possible

that 6-O-sulfotransferase-deficient patients lack a particular "signature" in HS that is important for the function of a specific HS proteoglycan (we do not know what specific HS proteoglycan is involved). This might lead to progressive macular disorganization and eventually to visual impairment.[105]

The enzyme responsible for the addition of sulfate to the 3 position of the glucosamine (3-O-sulfotransferase) and known to modulate the high-affinity binding of anti-thrombin III has been targeted in mice.[106] These mutant mice exhibit some growth retardation[106] and lack HS that possesses the anti-thrombin III binding sequence; however, they show normal coagulation and hemostasis[43] suggesting that other isoforms of the enzyme may be able to compensate for the 3-O-sulfotransferase 1 enzyme.[107] Alternatively, these sequences, although being shown to perform the anticoagulant function in vitro, may not perform the role in vivo and may be important for the endocytosis of anti-thrombin III by endothelial cells (N. W. Shworak, personal communication). Thus, other sequences might play this role in vivo, including those present in CS chains decorating thrombomodulin and known to enhance the binding to thrombin.[108,109]

4. Heparan Sulfate/Protein Interactions

HS has been shown to interact with hundreds of proteins. The vast majority of the work performed in this area has used the highly sulfated heparin as a model for HS.[110] Affinity column chromatography has been performed to isolate many different proteins, the majority of which bind via ionic interactions between the immobilized heparin and mostly lysine or arginine residues that are aligned in particular sequences known as "Cardin−Weintraub" sequences.[111] These sequences have become known as heparin-binding sequences and are used to identify those proteins or subdomains of proteins that potentially interact with the HS present in the surrounding biological environment. It should be noted, however, that many heparin-binding regions of proteins are not contiguous and may not involve these types of sequences; in some cases they have been shown to involve different regions of proteins that are positioned within the same three-dimensional areas due to the tertiary structure of the protein. Heparin, as discussed earlier, is a specific HS produced by mast cells that decorates the proteoglycan serglycin.[112] It has the same basic structure as HS except that it is produced mainly in a highly sulfated form that consists of large stretches of trisulfated disaccharides. Its heterogenic nature with respect to the length and charge of its HS chains, together with the idea that heparin affinity chromatography primarily acts as a cation-exchange column in many of the described protocols, has resulted in the misconception that many of the heparin protein interactions are "non-specific". On the contrary, there is now growing evidence suggesting that these interactions are indeed specific, and more investigators are moving away from heparin and utilizing HS as a more physiologic source of actively binding structures. The interactions among heparin/HS and the many different types of proteins have been reviewed extensively in other publications,[113,114] and only a few of those will be discussed here in more detail to provide the reader with some biologically relevant examples.

4.1. Cytokines

HS has been shown to interact with cytokines such as interleukin 5 (IL-5),[115] interleukin 6 (IL-6),[116] interleukin 8 (IL-8),[117] interleukin 10 (IL-10),[118] stromal cell-derived factor 1 (SDF-1),[119] tumor necrosis factor α (TNF-α),[120] monocyte chemo-attractant protein 1 (MCP-1),[121] and platelet factor 4 (PF-4).[122] These interactions serve to localize the cytokines to various extracellular compartments, as well as to control the activity of each cytokine. In the case of IL-8, the interaction promotes the activity of the cytokine, whereas, in the case of PF-4, the interaction inhibits the activity. In addition to localization, the potential interaction with HS in the extracellular matrix serves to set up and maintain gradients of these signaling molecules. This is important where cells are required to migrate through various tissues and organs. It has been suggested that the anchoring of these factors to the extracellular matrix of the bone marrow controls the activity of the cytokines and provides appropriate niches that subsequently control the differentiation of blood progenitor cells into the various cell types in the circulation.[123]

4.2. Growth Factors

The interactions between growth factors and HS have been studied extensively and, in particular, members of the fibroblast growth factor (FGF) family have been the focus of intense activities over the years. FGF-1, FGF-2, FGF-4, FGF-7, FGF-8, FGF-10, and FGF-18 have all been shown to bind to HS.[124] Some of the FGF receptors that bind these ligands such as FGF receptor-1 (FGFR-1), FGFR-2,[125] and FGFR-4[126] have also been shown to bind to HS possibly via heparin-binding sequences contained within the N-terminal IgG-like domain.[127] Furthermore, these interactions have been shown to vary and depend on the HS structure attached to different types of HS proteoglycans.[128] Heparin was the first HS shown to promote the signaling of FGFs.[129] This led investigators to propose various models to explain the phenomenon, one of which involves the heparin/HS, growth factor, and receptor coming together in a ternary complex.[130,131] Commercial heparin has been used to investigate this model,[132,133] as well as the interaction between HS and either FGF-1[134] or FGF-2.[135] The minimal binding sequence of heparin for FGF-2 was shown to be a hexasaccharide,[135] whereas a tetrasaccharide with a similar sequence to that described for FGF-2 was the minimal heparinase protected sequence that bound FGF-1.[134] Further studies using heparin have shown that the smallest biologically active sequence required was a decasaccharide,[136] the longer chain length being necessary to bridge the physical distance between the growth factor and its cognate receptor. N-Sulfate and 2-sulfate groups present in this decasaccharide are important for growth factor binding, whereas the

6-sulfate groups play a key role in cell signaling events.[137] More recently, various investigators have started examining tissue-derived HS and have shown that, like heparin, these tissue-derived HS preparations can promote of FGF receptor signaling.[126,138] Notably, in some instances, they were shown to have more activity and specificity for different FGF receptor isoforms than heparin itself.[138] In these preparations, it is not known what the cellular or molecular source of the active sequences is, although this might be irrelevant to receptor signaling with the most important component being its localization to the cell surface in an appropriate topology.[139] Most of these efforts have concentrated on examining those sequences in heparin or HS extracted from whole tissue preparations since chemical quantities are relatively easy to obtain from these sources. However, these preparations exhibit a high proportion of batch-to-batch variability, which in turn provides tenuous links between HS structure and its activity.

Growth factors such as hepatocyte growth factor (HGF),[140] platelet-derived growth factors (PDGF),[141,142] heparin-binding epidermal growth factors (HB-EGF),[143,144] neuregulin-1,[145] vascular endothelial growth factors (VEGF),[146−148] and bone morphogenetic protein 7 (BMP-7)[149] have been shown to interact with HS either in isolation or as part of proteoglycans decorating the cell surface or the extracellular matrix. HGF is a potent stimulator of epithelial cell migration, which has led to it being referred to as scatter factor. It has been shown to interact with HS on cell surfaces[150] as well as free HS and dermatan sulfate[151] via interactions between the sulfate groups and certain charged residues in the N-terminus of the growth factor.[140] Sequences of HS that contain 6-sulfated, N-sulfated glucosamine and 2-sulfated iduronic acid residues are particularly important and a minimal binding sequence must be at least 5−6 disaccharides in length.[140] Such sequences can activate, via the Met receptor, the MAP kinase cell signaling pathway involved in cell migration.[140] The longer spliced forms of PDGF that include a stretch of basic amino acids encoded by exon 6 interact with HS proteoglycans in the extracellular matrix[141] via sulfated regions that have a high proportion of trisulfated disaccharides,[142] whereas HB-EGF interacts with HS on the cell surface.[143] The alternatively spliced form of neuregulin-1 that interacts with heparin via the Ig-like domain has a preference for oligosaccharides that are rich in N-sulfation, although shorter sequences including disaccharides have been shown to interact quite effectively.[145] The precise structures of HS that are involved in these interactions remain to be elucidated but their differential partition either on the cell surface or in the extracellular matrix suggests that the structures may be specific to their location.

The HS attached to domain I of perlecan is important for the binding of growth factors such as FGF-2,[152] FGF-1,[153] FGF-9, the homodimer long chain forms of PDGF type A and the shorter type B, and HGF (Figure 4). Furthermore, the growth factor binding ability along with cell binding and antibody binding activity of perlecan HS has been shown to

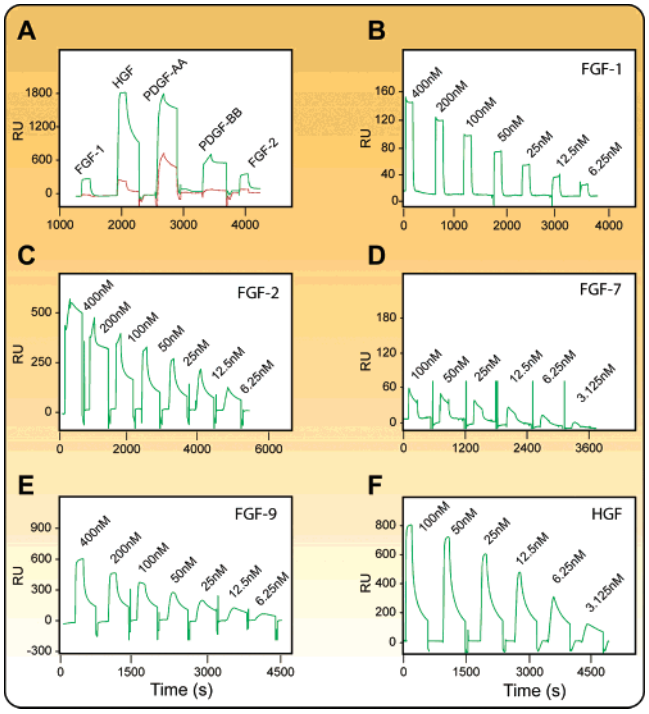


Figure 4. Surface plasmon resonance analysis of perlecan binding to various growth factors. Perlecan isolated from endothelial cells was biotinylated and bound to a streptavidin BIAcore chip surface. Growth factors analyzed include FGF-1, FGF-2, FGF-7, FGF-9, hepatocyte growth factor (HGF), and PDGF-AA and PDGF-BB, as indicated. The various growth factors were run over the chip surface at the concentrations indicated. The continuous green line in panel A indicates the binding of the growth factors to the intact proteoglycans, whereas the broken red line indicates the binding seen after treatment with heparitinase (heparinase III) to digest the HS chains.

be variable and depends on the cell source.[154] This property is also dependent on specific substructures of the HS chains.[155]

The VEGF family of growth factors consists of at least six-members: some have been alternatively spliced resulting in segments of protein being inserted that confer a basic charge in that region. This leads to the localization of these longer VEGFs to the extracellular matrix.[156] The VEGFs are linked covalently as dimers and exert their biological effects by binding to either of two cell membrane tyrosine kinase receptors, VEGFR1 (Flt-1) or VEGFR2 (Flk-1/KDR), both of which are expressed on endothelial cells.[157] Although VEGFR1 has a higher affinity for VEGF, it is the VEGFR2 that appears to be more important in the mitogenic and angiogenic responses of endothelial cells.[158] The exact role of VEGFR1 still remains to be determined. Mice that lack the VEGFR1 gene die early in utero due to the failure of the vasculature to become organized,[159] whereas VEGFR2 knockout mice do not have mature endothelial cells.[160]

HS proteoglycans are thought to play important roles in the binding of VEGFs to their cell surface receptors and subsequent signaling events. For example, exogenous heparin can rescue the proliferative response of cells exposed to VEGF that had been previously rendered refractile by incubation with heparinase.[161] On the other hand, HS proteoglycans

in cell membranes and the extracellular matrix are key modulators of VEGF signaling to endothelial cells. To date, glypican-1 is the only cell surface HS proteoglycan known to enhance the binding of VEGF-A_{165} to its receptors by binding the growth factor via its HS chains.[162] Perlecan, the major HS proteoglycan of most basement membranes binds the longer alternatively spliced form of VEGF-A_{189}, whereas the shorter VEGF-A_{165} isoform is not bound unless it is first complexed with VEGFR2. Interestingly, the form of VEGF-B that contains a heparin-binding site, VEGF-B_{164}, does bind to HS attached to endothelial perlecan (unpublished results). The binding of VEGF to HS has been shown to be important in vivo in the developing mouse vasculature where localized forms of the growth factor are essential for successful branching morphogenesis. Abnormal expression and distribution of the various heparin-binding or non-heparin-binding forms of VEGF result in significant changes in vessel length and number, suggesting that the balance between diffusible and bound forms of VEGF and the type of HS present in the extracellular environment has a critical role in tissue patterning.[146] The idea that the distribution of HS in the tissues is acting as a "growth factor tethering" agent that subsequently drives and guides the development of tissue and organs is supported by the reported interactions between FGF-10 and HS, and its importance in lung morphogenesis.[163] This hypothesis may also explain the intriguing results obtained from the 2-O-sulfotransferase knockout mouse model that shows a total failure of renal development.[164] The distribution of the various types of HS affects growth factor gradients and acts as a template upon which tissues and organs may develop. This may indeed represent a major role of HS and may occur with other HS-binding growth factors.

4.3. Lipases, Proteases, and Protease Inhibitors

HS has been shown to control the activity of lipases and proteases by directly binding to enzymes. An example is provided by HS/lipoprotein lipase interaction on the surface of endothelial cells where it has been postulated to be important in the binding and clearance of lipoproteins from the circulation.[165] It was shown that a decasaccharide of HS made up of repeating trisulfated disaccharides has a high affinity for binding to lipoprotein lipase.[166] HS is also thought to be important in the clearance of the lipoproteins via cell binding and uptake of apolipoprotein E.[167]

The generation of a thrombus at a site of injury or inflammation requires the successful conversion of inactive proenzymes to active forms that themselves further convert inactive enzymes in an efficient cascade fashion. The end result is the formation of fibrin, which is the major structural component of a thrombus, from the serum protein fibrinogen. HS has been hypothesized to play an important role in the control of this cascade via its interaction with the serine protease inhibitor anti-thrombin III, which is able to inhibit both Factor Xa, which converts prothrombin to thrombin, and thrombin (also known as Factor IIa), which converts soluble fibrinogen into fibrin monomers, which subsequently become cross-linked.[114,168] HS binds anti-thrombin III via a particular sequence present mostly in heparin and known to involve a pentasaccharide sequence that has a central trisulfated glucosamine residue that is critical for the interaction. Once bound to HS, the inhibitor undergoes a change in its tertiary structure that enables it to bind and block Factor Xa activity.[169] The successful inhibition of thrombin requires HS sequences longer than pentasaccharides, because HS binds both thrombin and anti-thrombin III and acts as a template where enzyme and inhibitor come in close proximity enabling an efficient interaction.[169] Once this occurs, the affinity of anti-thrombin III for the HS is reduced and the complex can be released into the circulation.[114] The longer HS sequences important for the inhibition of thrombin comprise at least eight disaccharides and must contain the anti-thrombin III binding pentasaccharide within their sequence.[170]

Other enzymes such as the metalloproteinases and their inhibitors also bind to HS. In fact, heparin-affinity chromatography has been a popular mode of purifying these enzymes from complex biological fluids, and it has been speculated that the interaction between matrix metalloproteinase 13 (MMP-13) and heparin has physiological relevance in that it may control the enzyme's location and activity.[171,172] MMP-7 has also been shown to bind to HS present in the extracellular matrix,[173] as has one of the inhibitors of this family of enzymes, tissue inhibitor of metalloproteinase 3 (TIMP-3).[174,175] However, it is of interest to note that stromelysin (MMP-3), one of the major metalloproteinases that cleaves the protein core of perlecan, does not bind heparin−Sepharose.[152] Collectively, the studies summarized above underscore a principal function of HS and specific microsequences within the HS polymer in determining the fate of various proteases and their inhibitors.

4.4. Extracellular Matrix Proteins

HS has been shown to interact with various extracellular matrix proteins including fibronectin,[176] laminin,[177] thrombospondin,[154,178,179] types I, II, and IV collagen,[180] type V collagen,[154,181] type VI collagen,[182] type XIII collagen,[183] and the C-terminal fragment of type XVIII collagen, endostatin.[184] The interaction of HS with endostatin relies on the presence of 6-sulfates in the sequence to a greater extent than the corresponding presence of 2-sulfates,[184] and this interaction has been shown to be inhibited by dodecasaccharides but not shorter sequences that bind FGF-2. This binding of endostatin by HS is important for its anti-angiogenic function.[185]

HS chains, either free or covalently attached to perlecan protein core, interact with another extracellular matrix protein, the proline arginine-rich and leucine-rich repeat protein (PRELP).[186] HS sequences of approximately seven to eight N-sulfated disaccharides in length, where some 2-sulfation has occurred on iduronic acid residues, attach to the heparin-binding region in the C-terminal portion of fibronectin.[176] HS bound to cell surface proteoglycans can also bind to the same site, and this interaction is thought to modulate the interaction between the cell-binding

activity of fibronectin and the integrins on the cell surface.[187,188] The HS sequence that was shown to have high affinity for the heparin-binding sequence in the G-domain of laminin-1 was a HS of six trisulfated disaccharides.[177] The interaction between HS and laminin could be important in determining the integrity of basement membranes,[189] as well as in modulating laminin-derived growth activity for neuronal cells distinct from its cell adhesive capacity.[190] A reduction in HS in basement membrane has been noted in diseases of the kidney glomerular basement membrane such as nephritis[191] and diabetic nephropathy.[192] Particular sulfation patterns of HS have been shown to change in experimental skin wounds. Specifically, an increase in HS sulfation is associated with an integral and completely healed basement membrane.[193]

The HS sequences that interact with and bind to thrombospondin-1 have been found to contain high proportions of di- and trisulfated disaccharides, the minimal binding length being a decasaccharide.[178] The presence of some of these thrombospondin-1-binding sequences in the HS attached to endothelial cell-derived perlecan is further supported by the observation that the major pericellular molecule involved in the HS-dependent interaction with thrombospondin is perlecan.[154,179] This interaction with the HS attached to perlecan may also play an important role in the binding of sickle cell erythrocytes to the endothelium[194] and may play a significant role in the attachment of hematopoietic progenitor cells to the extracellular matrix in the bone marrow niche.[195]

The interaction between HS and collagen V plays an important role in the modulation of cell adhesion to the substratum.[181] The binding of Schwann cells to the cell adhesive site present in the N-terminal domain of the α4 chain of collagen V is inhibited by the addition of heparin, suggesting an important role for HS in this interaction. Furthermore, Schwann cell adhesion can be modulated by the interaction between HS isolated from the cell surface syndecan-3 and collagen V.[196] Interestingly, the same region of collagen V also reacts with HS from both glypican-1 and perlecan,[197] suggesting that both integral membrane HS proteoglycans, as well as matrix-associated HS proteoglycans, can provide similar adhesive signals. Glycosaminoglycans other than HS have been shown to bind to collagen V. For example, chondroitin sulfate oligosaccharides bind to collagen V, with octasaccharides or larger containing a high proportion of disulfated disaccharides having the highest affinities.[198] It has been suggested that these structures also may play an important role in the regulation of cell adhesion to extracellular matrixes.[198]

4.5. Structural Analysis

The heterogeneous nature of HS chains both in length and charge, together with the low levels usually present in most biological samples, has made the detailed analysis of their structure very challenging. Compositional analysis has taken advantage of a group of bacterial endoglycosidases and chemical treatments that depolymerize the HS chains into their constituent disaccharides. These disaccharide fractions can then be analyzed by conventional electrophoresis using either polyacrylamide[199,200] or agarose,[201−203] together with fluorescent tagging of reducing ends of the HS chain,[200] or HPLC methods[204] followed by a comparison with standards. Mass spectrometric (MS) methods have successfully been applied to glycosaminoglycan analysis. Due to the presence of sulfate and hexuronic acid in these molecules, negative ion mode has usually been the method of choice for mass spectrometric analysis. Historically, fast atom bombardment and liquid secondary desorption/ionization techniques for analyzing native HS or depolymerized HS[205] have been substituted with negative ion electrospray (ES) mass spectrometry.[206,207] Even matrix-assisted laser desorptive ionization time-of-flight (MALDI-TOF) mass spectrometry methods have been successfully used, including coupling of the negatively charged HS fragments to positively charged polypeptides enabling detection in positive mode.[133,208] The combination of mass spectrometry and chromatography for analysis of HS digests provides additional improvement of the analysis since it enables isomeric oligosaccharides to be differentiated. While normal phase amino columns have successfully been employed for this type of analysis,[209] recent progress with reversed phase chromatography together with ion-pairing of glycosaminoglycan derivatives with hydrophobic amines in the mobile phase shows promise.[210,211]

The molecular masses of the disaccharides that constitute a HS chain vary from 378 to 576 Da: 378 Da for an unsulfated hexuronic acid linked to acetylated unsulfated hexosamine, 416 Da for an unsulfated hexuronic acid linked to an N-sulfated hexosamine, 458 Da for a monosulfated, acetylated disaccharide, 496 Da for a disulfated disaccharide composed of an N-sulfated hexosamine, 538 Da for a disulfated acetylated disaccharide, and 576 Da for the fully trisulfated disaccharide. A combination of liquid chromatography and ES MS can be used to determine the structure of some of these disaccharides, and by including MS/MS fragmentation in the analysis, we can distinguish between isomeric disaccharides. An example of this type of analysis is shown in Figure 5, where the two disaccharides with a molecular mass of 458 Da are shown. These disaccharides differ in the position of the sulfate group and Figure 5A shows the MS/MS analysis of the disaccharide with the sulfate in the 6 position of the *N*-acetylglucosamine (IIA), whereas Figure 5B shows the same analysis performed on the disaccharides with the sulfate attached to C-2 of the hexuronic residue (IIIA). A comparison of these two chromatograms clearly indicates the differences in the fragmentation patterns obtained from the two disaccharides. The Y1 fragment (*N*-acetylglucosamine-6-S) is one of the major peaks in the IIA chromatogram whereas the B1 fragment (hexuronic acid-2-S) can be seen in the chromatogram obtained from IIIA. These peaks are diagnostic for their particular disaccharide structures. If this type of analysis is linked with incubations with the various bacterial heparinase enzymes that have been shown to be specific for certain cleavage sites, we will be able to obtain data

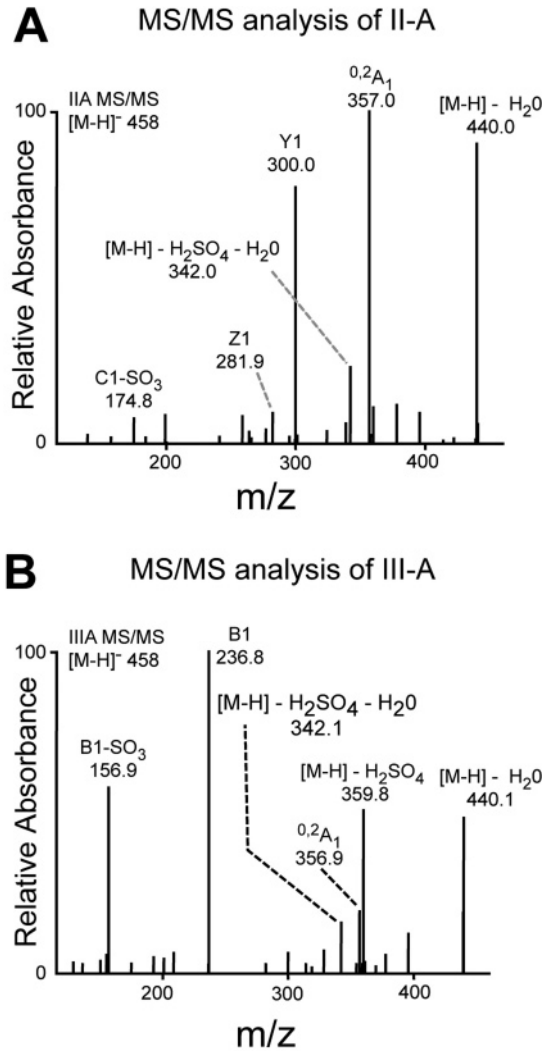


Figure 5. MS/MS analysis of isomeric HS disaccharides. HS disaccharide standards, IIA or IIIA, with identical molecular masses of 458 Da differ in the position of the sulfate group. Panel A shows MS/MS analysis of the disaccharide with the sulfate in the 6 position of the *N*-acetylglucosamine (IIA). Panel B shows the same analysis performed on the disaccharides with the sulfate attached to C-2 of the hexuronic residue (IIIA). A comparison of these two chromatograms clearly indicates the differences in the fragmentation patterns obtained from the two disaccharides. Note that the Y1 fragment (*N*-acetylglucosamine-6S) is one of the major peaks in the IIA, whereas the B1 fragment (hexuronic acid-2-S) can be seen in the chromatogram obtained from IIIA. The two isomeric disaccharides were reduced in 20 μL of 0.25 M sodium borohydride in 25 mM sodium hydroxide and desalted using cation-exchange resin (AG50W-X8, Biorad) contained within mini-spin columns (C_{18} ZipTip, Millipore). After elution, they were dried, redissolved in H_2O, and purified using graphitized carbon that had been placed in another mini-spin column. Bound disaccharides were eluted with 0.1% trifluoroacetic acid in 40% acetonitrile, dried, and redissolved in a small volume of H_2O. Liquid chromatography (LC) MS was performed on the samples by passing them through a column consisting of graphitized carbon (5 μm particles, Thermo Electron), eluting them with acetonitrile, and injecting them directly into an LCQ-XP+ Thermo-Finnigan system (San Jose, CA) using the standard electrospray interface with a capillary voltage of 3.5 kV and a temperature of 380 °C. The mass spectrometer was scanned from *m/z* 200 to 800 followed by a data-dependent MS^2 scan of the most intense ion from the previously recorded full scan spectrum.

that confirm the structures of known biologically active sequences.

To catalog the structures of HS that will be forthcoming from the increase in research activity in the mass spectrometry area, a nomenclature and searchable database would be both useful additions to the area and a tool for researchers to utilize. A nomenclature has been suggested based on either a number or letter with the prefix "−" for glucuronic acid-containing and "+" for iduronic acid-containing disaccharides for each of the 32 possible disaccharides that make up HS.[133] There are various databases that have been built over the years that contain the common N-linked, as well as some of the O-linked, oligosaccharide structures attached to protein cores, but none has included the structures of glycosaminoglycans and HS very successfully. The GlycoSuite database is a fully curated database that was initiated in 1999 and made available for first use in 2000.[212] It has recently been commercialized and is available under license. It contains many structures of glycosylation sequences that have been identified attached to proteins by either oxygen to serine or threonine (O-linked) or nitrogen to asparagine (N-linked).[213] However, it does not have many glycosaminoglycan structures, and hence HS, but it is expected to expand in this area as these structures are generated over the next years.

5. Involvement of Heparan Sulfate in Various Disease Processes

The role of HS in various biological processes such as embryonic development has been established in studies that have been covered in section 3 of this review. As a consequence of some of the knockout studies, light has been shed on the role of HS in various pathological situations. One such example is the association of the *ext* family of genes, also known as exostosins (glycosyltransferases that synthesize HS), with a dominantly inherited disease involving abnormal development of skeletal tissue, most notably the presence of cartilaginous tumor-like tissue in some of the joints due to unchecked differentiation of chondrocytes into bone cells.[21] This disease has been termed exostosis[214] due to the presence of bony tissue outgrowths on the ends of the long bones.[86]

It has been hypothesized that the spread of cancer throughout the body involves the removal of HS in the tissue, particularly basement membranes, which facilitates the movement of the cells to distant sites.[215−217] Apart from invasion and metastasis of tumor cells, other key issues in cancer biology involve the understanding of factors that control tumor cell proliferation and the identification of extracellular cues that regulate the signal-transducing repertoire of cancer cells. Among these factors, HS proteoglycans occupy a central role because of their ability to bind growth factors and modulate their biological activities.[6,8,110,218,219] Perlecan is a modular proteoglycan that participates in the formation and maintenance of basement membranes[220−222] and in the regulation of the vascular response to injury.[223] The protein modules of perlecan have striking homology to polypeptides involved in lipid uptake, growth

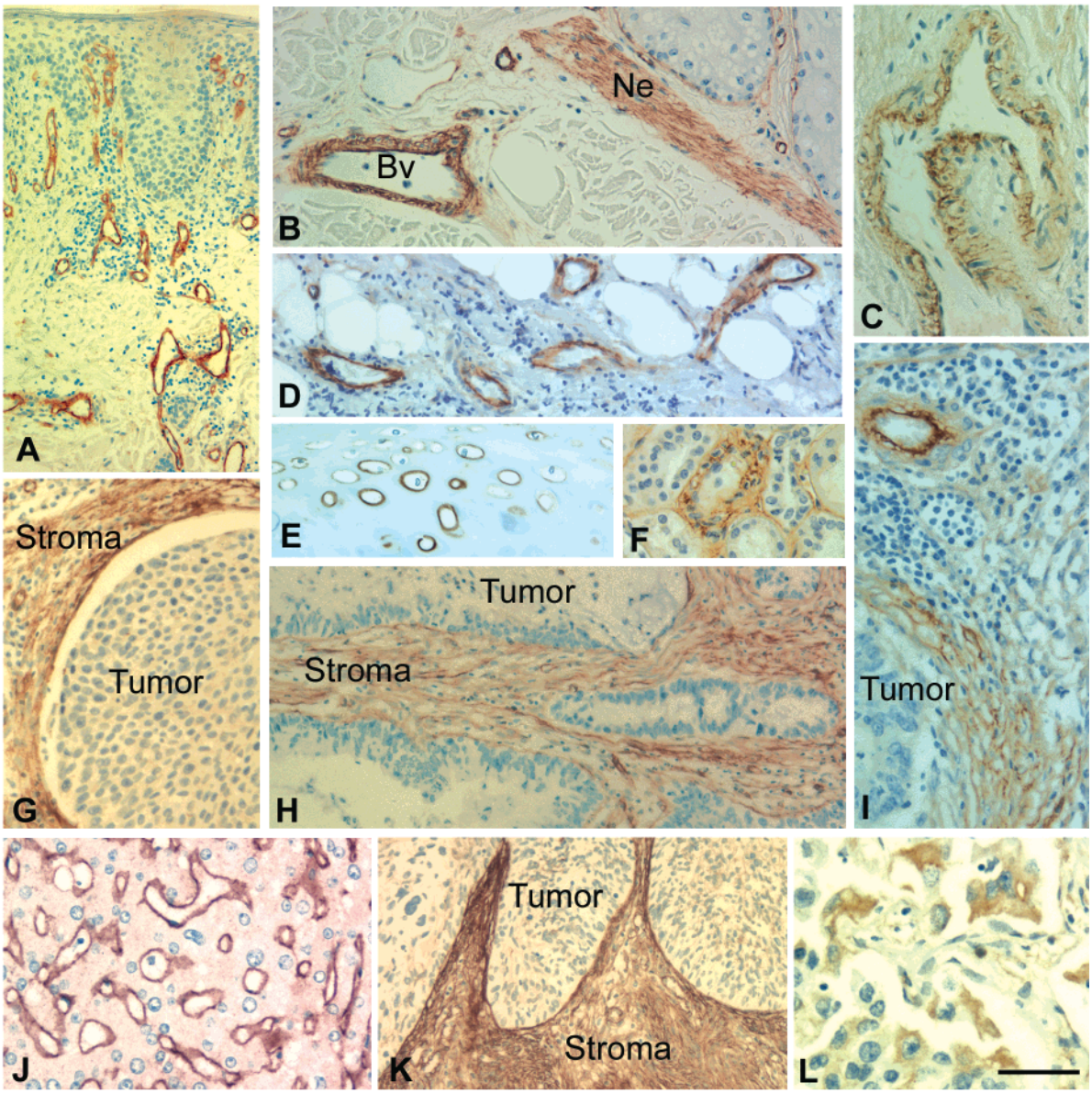


Figure 6. Gallery of photomicrographs of various normal and neoplastic human tissues immunoreacted with an antibody directed toward the protein core of perlecan. Positive immunoreactivity is shown by the dark brown staining. In panel A, normal skin shows specific blood vessel staining in the dermis. Panel B shows subcutaneous soft tissue with immunoreactivity in a small blood vessel (Bv) and a myelinated nerve (Ne). In panel C, a midsize vein shows staining around smooth muscle cells. In panel D, fibroadipose tissue shows selective staining of microvasculature. Panel E shows bronchial cartilage; notice that the pericellular matrix of chondrocyte is stained. The perlecan in the cartilage matrix is masked and can be visualized after digestion with chondroitinase ABC. Panel F shows immunoreactivity in the basement membrane of renal tubules. Panel G shows transitional cell carcinoma of the urinary bladder. Notice that perlecan is primarily localized to the stroma. The remaining panels show (H) colon carcinoma, (I) breast carcinoma, (J) astrocytoma, (K) ovarian carcinoma, and (L) poorly differentiated renal cell carcinoma. Scale bar = 100 μm.

control, cell−cell interactions, and adhesion. Its highly refined molecular architecture,[224−226] coupled with its ubiquity, suggests that perlecan may perform key biological functions during ontogeny, tissue remodeling, or transformation.[8,227] Lack of perlecan causes embryonic lethality with severe cephalic and cartilage abnormalities.[228,229] Although basement membranes can develop in the absence of perlecan, perlecan-deficient mice succumb to intra-pericardial hemorrhages at day 10.5, when vasculogenesis is prominent and intraventricular pressure rises,[229] and if they survive, the perlecan-null animals exhibit a high incidence of cardiac outflow malformations.[230] In mature tissues, perlecan is a major HS proteoglycan secreted by endothelial cells and present in both vascular and avascular tissues (Figure 6A−E). Perlecan is potent inhibitor of smooth muscle cell proliferation[231−234] and is a major candidate for the FGF-2 low-affinity receptor; that is, addition of exogenous perlecan can restore high-affinity binding of FGF-2 to its receptor in HS-deficient cells,[235] along with stimulating cell growth in similar cells transfected with appropriate FGF receptors.[155] FGF-2 binds to the HS chains attached to the N-terminal domain I,[155] and the availability of this powerful angiogenic factor is modulated by the concerted action of heparanase and proteases.[152] Perlecan is highly enriched in various tumorigenic cell lines[236,237]

and tumors, including melanomas and colon and breast carcinomas,[238,239] where it is expressed primarily by the connective tissue stroma (Figure 6G,H,K); however, in certain poorly differentiated carcinomas perlecan epitopes are also found within the cytoplasm of tumor cells (Figure 6L).

Blocking endogenous production of perlecan suppresses autocrine and paracrine functions of FGF-2 and impairs tumor growth and invasion.[240−242] Perlecan also binds to FGF-7[242,243] and is required for functional activation of the FGF-7 receptor.[244] In addition, perlecan protein core binds several extracellular matrix molecules[245] and acts as either an adhesive or a counter-adhesive molecule.[154,246−248] Angiogenesis is one of the most important events in tumor progression[234,249,250] and is greatly influenced by cell−matrix interactions taking place at the surface of endothelial cells and at tumor−matrix boundaries.[251] Recently, the C-terminal domain V of perlecan was shown to be a potent inhibitor of angiogenesis.[252] This 85-kDa fragment, named "endorepellin" to designate its anti-endothelial cell activity, inhibits angiogenesis and interferes with various aspects of endothelial cell biology including migration, adhesion, and tube formation,[253] indicating that endorepellin might represent a novel biological tool against cancer.[254]

Similarly, other HS proteoglycans have the ability to regulate growth and tumorigenesis. An example is provided by glypican.[255,256] For example, mutations in glypican-3 cause the Simpson−Golabi−Behemel syndrome, an X-linked disorder characterized by prenatal as well as postnatal overgrowth. This syndrome is quite complex and includes macroglossia, cleft palate, syndactyly and polydactyly, cystic and dysplastic kidneys, and congenital heart defects. The consensus is that glypican-3 is a negative regulator of insulin-like growth factor II (IGF-II) insofar as similar phenotypes are observed in mice overexpressing IGF-II or mice that have a targeted mutation of the IGF-II receptor.[255,256] Given this specific growth regulatory ability of glypicans, it is not surprising that this gene product has been implicated in the pathogenesis of cancer. The expression of glypican-1, for example, is markedly enhanced in pancreatic carcinomas and regulates growth factor activity in these cells.[257] Specifically, endogenous suppression of glypican-1 gene expression using antisense oligonucleotides also blocks the mitogenic response of cultured pancreatic cancer cells to FGF-2 and heparin-binding EGF-like growth factor.[257] Glypican-3, on the other hand, behaves as an oncofetal protein; that is, it is expressed during embryonic development but much less in adult organs. Indeed, glypican-3 is not expressed in adult liver, but its expression is highly increased in hepatocellular carcinomas.[256] Overall, oncofetal proteins do not tend to play a direct role in tumorigenesis, but rather they play a role as oncofetal markers. Thus, the role of glypican-3 in cancer needs to be further elucidated.

Research investigating the hypothesis that in cancer the amount of HS in the basement membrane and connective tissues is reduced by the specific action of enzymes has led investigators to the discovery, characterization, and cloning of an enzyme called heparanase[258,259] that is responsible for HS turnover, most probably via a lysosomal route.[55,260] Interestingly, overexpression of heparanase leads to enhanced angiogenesis and mammary gland morphogenesis suggesting that an increase in the turnover of HS in the targeted tissues would result in a concomitant disruption of the epithelial basement membrane.[261] The mice also demonstrate an increase in the rate of hair growth and decrease in body weight due to a reduced appetite.[261] In addition to the above-mentioned properties, heparanase may also play an important "processing" role in the production of the specific HS, heparin, from serglycin in mast cells.[262]

A reduction in the amount of HS present in tissues has been achieved by modifying the perlecan gene in animals so that it produces a truncated protein core. In support of the important role this form of HS plays in cancer growth, a reduction in the growth of FGF-2-reliant tumors was demonstrated in these animals, along with a reduction in the rate of wound healing and angiogenesis.[263] This supported previous work in a system using colon carcinoma cells that produced significant quantities of perlecan in the pericellular environment[236] and the subsequent reduction of the expression of perlecan, and hence its HS, in these cells by the use of antisense technology against the protein core of the HS proteoglycan. Cancer cells transfected with the antisense construct had slower growth rates of cells in vitro and in vivo in tumor xenografts.[242] Other diseases that have been shown to be associated with a decrease in the amount of HS include the nephrotic syndrome known as Denys−Drash disorder in which the levels of secreted glycosaminoglycans and proteins in the urine are elevated due to a malfunction (loss of the anionic HS) in the filtering capacity of the glomerular basement membrane.[264]

There are also genetic diseases involving the accumulation of HS within cells. The most notable of these is a family of diseases resulting from the abnormal breakdown and turnover of HS by cells. Together with those diseases that involve the abnormal turnover of other GAG types, they are termed "mucopolysaccharidoses" (MPS) or lysosomal storage disorders. The HS-related diseases are the result of mutations in genes of lysosomal enzymes involved in the intracellular degradation of HS chains. The disorders are classified depending upon the enzyme(s) that is involved and are often identified after birth as patients develop symptoms such as skeletal and neuronal abnormalities due to accumulation of HS fragments within the lysosomes. This pathological processing and intracellular storage eventually leads to an abnormal secretion of HS fragments in the urine of affected patients. These syndromes are complex in their manifestation because various mutations within the same gene can lead to different types, and it has been suggested that there are over 40 different types of MPS.[265] For example, MPS type II is caused by a mutation in the iduronate-2-sulfatase enzyme, the activity of which varies depending upon where the mutation occurs. This varia-

tion in activity often leads to differences in severity of the disease among a patient population.[266] The MPS disease process is not well understood—particularly the degenerative brain pathology that appears soon after birth and progressively gets worse. Apart from underlying the importance of HS in brain and neural development in the fetus, it is clear that turnover of HS in the growing brain of the developing human is important for correct adult brain development. One might speculate that HS is turned over by the neural cells as they differentiate and grow. When the lysosomal machinery of the neural cells is slowed by the accumulation of the undigested HS, further neural cell development is halted resulting in severe retardation. It is hoped that by the use of enzyme replacement therapy, either prophylactically or by gene therapy, that many of the deleterious side effects of these disorders can be overcome.[267]

Other diseases in which HS has been hypothesized to play a role include atherosclerosis, where the presence of HS has been shown to control the proliferation of the smooth muscle cells, which are responsible for a large proportion of the extracellular matrix that is produced in this disease.[233] Smooth muscle cell proliferation has also been shown to be controlled by perlecan via the activation of a tumor suppressor gene known as *PTEN*,[268] which acts to decrease the activity of focal adhesion kinase via the *ERK 1* and *2* pathway.[269] The presence of HS in the arterial wall has also been shown to be associated with reduced blood sugar levels and the reduced presence of atherosclerotic lesions in diabetic monkeys.[270]

The entry of viruses into cells involves the use of HS proteoglycans on the surface as co-receptors that act in concert with the specific binding epitopes. In the case of herpes simplex virus type 1 (HSV-1), this interaction has been shown to be between basic amino acids in the glycoprotein C on the viral envelope and the HS on the cell surface;[271] this interaction involves specific HS sequences containing additional sulfate groups at certain positions along the HS chain.[272,273] Recently, HS has been hypothesized to play a role in the pathology of transmissible spongiform encephalopathies (including BSE) via an interaction with the prion protein that may control the presence of the insoluble aggregates in neural tissues.[274]

Heparan sulfate may also have a role in the pathology of diabetes where a thickening of the glomerular basement membrane causes the failure of the filtering mechanism of the kidney resulting in the presence of plasma proteins in the urine. The loss of the function of this basement membrane has been hypothesized to be due to a decrease in the amount of sulfation present on the HS attached to perlecan and possibly other proteoglycans within the basement membrane.[275] This phenomenon may not to be restricted to the basement membrane of the kidney because it has been shown that HS isolated from the liver of diabetic animals also has reduced sulfation levels.[276] Degenerative changes in neural tissue are also associated with diabetes due to the abnormal thickening of basement membranes. These neuropathological changes have been likened to those seen in Alzheimer's disease where plaque material builds up around neurons, thereby interfering with correct neuronal signaling. This plaque material has been shown to be composed of β-amyloid protein fragments and HS[277] that may be attached to either perlecan, type XVIII collagen,[278] or agrin.[279] It has been suggested that HS chains bind and precipitate the fragments thereby preventing their clearance from the system,[280] a process dependent upon the HS sulfation pattern.[281] This disease process may also be promoted via the interaction with HS and the subsequent inhibition of the enzyme BACE-1 (β-site Alzheimer's associated protein cleaving enzyme), which degrades the amyloid protein[282] resulting in a build up of amyloid that promotes plaque formation. The precise role of HS in this disease process has not been clearly elucidated and awaits further investigation.

6. Heparan Sulfate Therapeutics

The idea that HS and structures modeled on it may be developed as therapeutic agents for various clinical applications has been around for many years but has recently gathered pace due to the additional activities that have been identified in some commercial HS preparations.[283] Heparin has been used clinically and marketed commercially since the beginning of the 20th century. It is very difficult to obtain precise information on how these are manufactured due to this information being proprietary. However, some of the methods, including those that remove cellular and protein components are common and relatively well understood. Low molecular weight (LMW) heparins have been developed for clinical use due to the demand by clinicians, who wanted a product that was more reliable and controlled in clinical situations. The dosage regime of heparin can vary widely among patients and depends on many factors, such as body weight; however other factors are still very important and remain poorly understood. If the dosage is not monitored carefully, it may lead to excessive bleeding or other adverse events such as heparin-induced thrombocytopenia, which has been suggested to be caused by the generation of broadly specific antibodies in the patient's circulation.[284] LMW heparins have been manufactured by first performing a controlled fragmentation of the longer polysaccharide chains, usually by chemical means (e.g., limited nitrous acid digestion or β-elimination). However, cleavage with heparinase enzymes has also been used, followed by separating the fragments by gel filtration chromatography. This has resulted in "heparin preparations" that have more controlled biological activities, although it has been shown that the way in which these molecules are manufactured affects their pharmacology.[285] The literature on the use of LMW heparins is vast and, as such, cannot be covered totally here but has been covered by others in more detail.[286−288] The anticoagulant activity of heparin is due mostly to its interaction with the protease inhibitor of the coagulation cascade, anti-thrombin III, which reduces the degradation of fibrin by promoting the inhibition of

the protease thrombin. The anti-thrombin III sequence is well characterized (see earlier section), and after pioneering work by a team of Italian and French scientists in the mid 1980s to early 1990s,[289,290] a totally synthetic approach to the synthesis of this pentasaccharide has resulted in the generation of the anti-thrombin III binding pentasaccharide.[291] This heparin fragment has been approved for use in the dissolution of thrombi after major orthopedic surgical procedures.[292,293] This synthetic approach has evolved to where both heparin and HS structures can be constructed by joining together sequences made up of their known disaccharide units.[294−296] The long term goal of this research is to progress the chemistry to a stage that allows HS and other glycosaminoglycan structures to be automatically synthesized. These structures could then be attached to microarrays and used to discover and study potential interactions between HS and other cell effector molecules.[297,298] It will also enable researchers to synthesize suitable quantities of the structures under appropriate guidelines such as Good Manufacturing Practice that will provide them with a high-quality and reliable product for clinical development.

As discussed earlier, we are now discovering the structures present in HS that have different biological activities than those observed in heparin. Therapeutic HS mimetics have been available commercially for many years. For example, dextran sulfate[299] and other sulfated glucose-derived polymers[300] have been developed and shown to have anticoagulant activity similar to that seen in preparations of heparin. Pentosan polysulfate is another synthetic sulfated polymer that has been shown to have anticoagulant[301] as well as anti-viral[302] activity and has been investigated as a potential therapeutic approach to control the breakdown of cartilage tissue seen in osteoarthritis.[303,304]

A sulfated phosphomannose heparin mimetic known as PI-88, which has anticoagulant activity,[305] is being developed as an anti-proliferative agent and anticancer compound due to its ability to bind to some of the angiogenic growth factors, FGF2 and VEGF.[306] It is also a potent heparanase inhibitor,[307] supporting the idea that it could have a dual function as an antitumor agent: it would inhibit the neovascularization associated with malignancies and could also prevent the metastatic spread of cancers by blocking a key event in the metastatic process, that is, the degradation of HS in the basement membrane. The same compound may also be useful for other clinical applications including the control of excess smooth muscle cell growth seen in restenotic lesions of coronary blood vessels after injury due to intravascular procedures such as angioplasty and stent placement.[308] Other compounds that are under development for use in biological applications include a group of sulfated cyclic compounds that interact specifically with the growth factors, such as FGF-1, FGF-2, and VEGF, the activity of which is dependent upon the position of the sulfate groups and the length of the spacer group.[309] Thus, the extensive structural and molecular characterization accumulated in the past decades will undoubtedly lead to better therapeutics based on heparin and HS mimetics or naturally derived fragments of these charged biopolymers.

7. Conclusions and Perspectives

Heparan sulfate is involved in a disparate cadre of molecular interactions and affects normal development as well as various pathologies. From its original discovery as a "contaminant" preparation, the interest in HS structure and function has surged to increasingly high levels in chemistry, biology, and medicine. The effects of the elimination or misexpression of HS, either by altering the protein core of various HS proteoglycans or by abrogating the expression of one of the key enzymes involved in HS biosynthesis and secretion, are surprisingly selective for specific signaling pathways. Differentially modified domains of HS expressed at specific temporal and topographic stages can act as molecular identifiers for the recruitment and assembly of morphogens and other molecular cues. One of the most striking observations is that HS synthesized by mice with mutations of various HS-synthesizing enzymes (NDST-1, NDST-2, and C-5-epimerase) exhibit identical structural abnormalities in all the tissues analyzed.[91] These findings are counterintuitive and suggest that the HS biosynthetic machinery has the same organization in all of the organs and that the mutational effects on HS molecular structure in these animals are general. Are the minute changes in HS structure less critical for organogenesis? Is there any maternal compensation during embryonic life? Are the HS structural "signatures" more important for adult life? And, if so, what are the consequences of anomalous structures? Will new syndromes or diseases be causatively linked to HS abnormalities? The answer to some of these questions will certainly make this field of research increasingly exciting and will likely attract new research interest in HS.

Clearly, the importance of HS in biology, development, tissue remodeling, and pathology cannot be overemphasized. However, the biological mechanisms of action and molecular structures of HS need to be carefully and exactly elucidated from results using methods and model systems that potentially mirror the activities and situations seen in biology. To achieve the aim of cataloguing and identifying biologically active structures, the field has started to use mass spectrometry and structural databases. Additional tools that have been either synthesized or constructed and may play important roles in identifying these active structures include oligosaccharide libraries[310] and microarray analyses. Phage display technology has also been used to generate useful reagents that identify different structural components present in HS and complement those antibody probes already available.[311,312] These technologies can be combined to provide very useful and high-throughput systems that can potentially identify responsible sequences from the myriad of structures that appear to be present in biological systems. The field of "glycomics" is one that encompasses many of these technologies, and given that it is in its early phases of development, not only will it continue to provide new and interesting information on how

carbohydrates affect various biological activities of proteins, but it will also add to the already vast literature supporting the importance of glycosaminoglycans, such as HS, in biological signaling.

8. Acknowledgment

We are deeply indebted to S. Selleck for providing Figure 3 and the corresponding information, C.C. Reed for providing the cover figure and for help with the illustrations, C.C. Clark for critical reading of this manuscript, S. Knox for performing the surface plasmon resonance experiments, and N. Karlsson for help with mass spectral data analysis. The original work in the authors' laboratories were, in part, supported by a Faculty of Engineering Research Grant, University of New South Wales, Australian Research Council Linkage Grant LP0455407, and Discovery Grant DP0557863 to J.W., and NIH Grants RO1 CA39481 and RO1 CA47282 and Department of the Army Grant DAMD17-00-1-0425 to R.V.I.

9. References

(1) Lindahl, U.; Kusche-Gullberg, M.; Kjellén, L. *J. Biol. Chem.* **1998**, *273*, 24979.
(2) Lander, A. D.; Selleck, S. B. *J. Cell Biol*. **2000**, *148*, 227.
(3) Messmore, H. L.; Wehrmacher, W. H.; Coyne, E.; Fareed, J. *Semin. Thromb. Hemostasis* **2004**, *30*, 81.
(4) Linker, A.; Hoffman, P.; Sampson, P.; Meyer, K. *Biochim. Biophys. Acta* **1958**, *29*, 443.
(5) Hirschberg, C. B.; Robbins, P. W.; Abeijon, C. *Annu. Rev. Biochem.* **1998**, *67*, 49.
(6) Esko, J. D.; Lindahl, U. *J. Clin. Invest.* **2001**, *108*, 169.
(7) Esko, J. D.; Selleck, S. B. *Annu. Rev. Biochem.* **2002**, *71*, 435.
(8) Iozzo, R. V.; San Antonio, J. D. *J. Clin. Invest*. **2001**, *108*, 349.
(9) Zhang, L.; Esko, J. D. *J. Biol. Chem*. **1994**, *269*, 19295.
(10) Zhang, L.; David, G.; Esko, J. D. *J. Biol. Chem.* **1995**, *270*, 27127.
(11) Dolan, M.; Horchar, T.; Rigatti, B.; Hassell, J. R. *J. Biol. Chem.* **1997**, *272*, 4316.
(12) Costell, M.; Mann, K.; Yamada, Y.; Timpl, R. *Eur. J. Biochem.* **1997**, *243*, 115.
(13) Groffen, A. J. A.; Buskens, C. A. F.; Tryggvason, K.; Veerkamp, J. H.; Monnens, L. A. H.; Vandenheuvel, L. P. W. J. *Eur. J. Biochem.* **1996**, *241*, 827.
(14) Graham, L. D.; Whitelock, J. M.; Underwood, P. A. *Biochem. Biophys. Res. Commun.* **1999**, *256*, 542.
(15) Chen, R. L.; Lander, A. D. *J. Biol. Chem.* **2001**, *276*, 7507.
(16) Dong, S.; Cole, G. J.; Halfter, W. *J. Biol. Chem.* **2003**, *278*, 1700.
(17) Kitagawa, H.; Kano, Y.; Shimakawa, H.; Goto, F.; Ogawa, T.; Okabe, H.; Sugahara, K. *Glycobiology* **1999**, *9*, 697.
(18) Lidholt, K.; Fjelstad, M.; Lindahl, U.; Goto, F.; Ogawa, T.; Kitagawa, H.; Sugahara, K. *Glycoconjugate J.* **1997**, *14*, 737.
(19) Kitagawa, H.; Tanaka, Y.; Tsuchida, K.; Goto, F.; Ogawa, T.; Lidholt, K.; Lindahl, U.; Sugahara, K. *J. Biol. Chem.* **1995**, *270*, 22190.
(20) Sugahara, K.; Kitagawa, H. *Curr. Opin. Struct. Biol.* **2000**, *10*, 518.
(21) Duncan, G.; McCormick, C.; Tufaro, F. *J. Clin. Invest.* **2001**, *108*, 511.
(22) Wei, G.; Bai, X.; Gabb, M. M.; Bame, K. J.; Koshy, T. I.; Spear, P. G.; Esko, J. D. *J. Biol. Chem.* **2000**, *275*, 27733.
(23) Kim, B. T.; Kitagawa, H.; Tamura, J.; Saito, T.; Kusche-Gullberg, M.; Lindahl, U.; Sugahara, K. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 7176.
(24) Kim, B. T.; Kitagawa, H.; Tamura Ji, J.; Kusche-Gullberg, M.; Lindahl, U.; Sugahara, K. *J. Biol. Chem.* **2002**, *277*, 13659.
(25) Kusche-Gullberg, M.; Kjellén, L. *Curr. Opin. Struct. Biol.* **2003**, *13*.
(26) Aikawa, J.; Grobe, K.; Tsujimoto, M.; Esko, J. D. *J. Biol. Chem.* **2001**, *276*, 5876.
(27) Toma, L.; Berninsone, P.; Hirschberg, C. B. *J. Biol. Chem.* **1998**, *273*, 22458.
(28) Berninsone, P.; Hirschberg, C. B. *J. Biol. Chem.* **1998**, *273*, 25556.
(29) Guillen, E.; Abeijon, C.; Hirschberg, C. B. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7888.
(30) Jacobsson, I.; Lindahl, U.; Jensen, J. W.; Roden, L.; Prihar, H.; Feingold, D. S. *J. Biol. Chem.* **1984**, *259*, 1056.
(31) Crawford, B. E.; Olson, S. K.; Esko, J. D.; Pinhal, M. A. *J. Biol. Chem.* **2001**, *276*, 21538.
(32) Kobayashi, M.; Habuchi, H.; Yoneda, M.; Habuchi, O.; Kimata, K. *J. Biol. Chem.* **1997**, *272*, 13980.
(33) Pinhal, M. A.; Smith, B.; Olson, S.; Aikawa, J.; Kimata, K.; Esko, J. D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 12984.
(34) Rong, J.; Habuchi, H.; Kimata, K.; Lindahl, U.; Kusche-Gullberg, M. *Biochemistry* **2001**, *40*, 5548.
(35) Kobayashi, M.; Sugumaran, G.; Liu, J.; Shworak, N. W.; Silbert, J. E.; Rosenberg, R. D. *J. Biol. Chem.* **1999**, *274*, 10474.
(36) Habuchi, H.; Tanaka, M.; Habuchi, O.; Yoshida, K.; Suzuki, H.; Ban, K.; Kimata, K. *J. Biol. Chem.* **2000**, *275*, 2859.
(37) Hemmerich, S.; Lee, J. K.; Bhakta, S.; Bistrup, A.; Ruddle, N. R.; Rosen, S. D. *Glycobiology* **2001**, *11*, 75.
(38) Kitagawa, H.; Fujita, M.; Ito, N.; Sugahara, K. *J. Biol. Chem.* **2000**, *275*, 21075.
(39) Zhang, L.; Beeler, D. L.; Lawrence, R.; Lech, M.; Liu, J.; Davis, J. C.; Shriver, Z.; Sasisekharan, R.; Rosenberg, R. D. *J. Biol. Chem.* **2001**, *276*, 42311.
(40) Jemth, P.; Smeds, E.; Do, A. T.; Habuchi, H.; Kimata, K.; Lindahl, U.; Kusche-Gullberg, M. *J. Biol. Chem.* **2003**, *278*, 24371.
(41) Razi, N.; Lindahl, U. *J. Biol. Chem.* **1995**, *270*, 11267.
(42) Kusche, M.; Backstrom, G.; Riesenfeld, J.; Petitou, M.; Choay, J.; Lindahl, U. *J. Biol. Chem.* **1988**, *263*, 15474.
(43) Shworak, N. W.; HajMohammadi, S.; de Agostini, A. I.; Rosenberg, R. D. *Glycoconjugate J.* **2002**, *19*, 355.
(44) Duncan, M. B.; Chen, J.; Krise, J. P.; Liu, J. *Biochim. Biophys. Acta* **2004**, *1671*, 34.
(45) Liu, J.; Shworak, N. W.; Sinay, P.; Schwartz, J. J.; Zhang, L.; Fritze, L. M.; Rosenberg, R. D. *J. Biol. Chem.* **1999**, *274*, 5185.
(46) Mochizuki, H.; Yoshida, K.; Gotoh, M.; Sugioka, S.; Kikuchi, N.; Kwon, Y. D.; Tawada, A.; Maeyama, K.; Inaba, N.; Hiruma, T.; Kimata, K.; Narimatsu, H. *J. Biol. Chem.* **2003**, *278*, 26780.
(47) Chen, J.; Duncan, M. B.; Carrick, K.; Pope, R. M.; Liu, J. *Glycobiology* **2003**, *13*, 785.
(48) Liu, J.; Shriver, Z.; Blaiklock, P.; Yoshida, K.; Sasisekharan, R.; Rosenberg, R. D. *J. Biol. Chem.* **1999**, *274*, 38155.
(49) Edavettal, S. C.; Carrick, K.; Shah, R. R.; Pedersen, L. C.; Tropsha, A.; Pope, R. M.; Liu, J. *Biochemistry* **2004**, *43*, 4680.
(50) Edavettal, S. C.; Lee, K. A.; Negishi, M.; Linhardt, R. J.; Liu, J.; Pedersen, L. C. *J. Biol. Chem.* **2004**, *279*, 25789.
(51) Zhang, L.; Lawrence, R.; Schwartz, J. J.; Bai, X.; Wei, G.; Esko, J. D.; Rosenberg, R. D. *J. Biol. Chem.* **2001**, *276*, 28806.
(52) Iozzo, R. V. *J. Biol. Chem.* **1987**, *262*, 1888.
(53) Beavan, L. A.; Davies, M.; Couchman, J. R.; Williams, M. A.; Mason, R. M. *Arch. Biochem. Biophys.* **1989**, *269*, 576.
(54) Lark, M. W.; Culp, L. A. *J. Biol. Chem.* **1984**, *259*, 212.
(55) Goldshmidt, O.; Nadav, L.; Aingorn, H.; Irit, C.; Feinstein, N.; Ilan, N.; Zamir, E.; Geiger, B.; Vlodavsky, I.; Katz, B. Z. *Exp. Cell Res.* **2002**, *281*, 50.
(56) Bai, X.; Bame, K. J.; Habuchi, H.; Kimata, K.; Esko, J. D. *J. Biol. Chem.* **1997**, *272*, 23172.
(57) Morimoto-Tomita, M.; Uchimura, K.; Werb, Z.; Hemmerich, S.; Rosen, S. D. *J. Biol. Chem.* **2002**, *277*, 49175.
(58) Dhoot, G. K.; Gustafsson, M. K.; Ai, X.; Sun, W.; Standiford, D. M.; Emerson, C. P., Jr. *Science* **2001**, *293*, 1663.
(59) Ai, X.; Do, A. T.; Lozynska, O.; Kusche-Gullberg, M.; Lindahl, U.; Emerson, C. P., Jr. *J. Cell Biol.* **2003**, *162*, 341.
(60) Viviano, B. L.; Paine-Saunders, S.; Gasiunas, N.; Gallagher, J.; Saunders, S. *J. Biol. Chem.* **2004**, *279*, 5604.
(61) Wang, S.; Ai, X.; Freeman, S. D.; Pownall, M. E.; Lu, Q.; Kessler, D. S.; Emerson, C. P., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 4833.
(62) Bulik, D. A.; Wei, G.; Toyoda, H.; Kinoshita-Toyoda, A.; Waldrip, W. R.; Esko, J. D.; Robbins, P. W.; Selleck, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 10838.
(63) Herman, T.; Horvitz, H. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 974.
(64) Toyoda, H.; Kinoshita-Toyoda, A.; Fox, B.; Selleck, S. B. *J. Biol. Chem.* **2000**, *275*, 21856.
(65) Lin, X. *Development* **2004**, *131*, 6009.
(66) Kirkpatrick, C. A.; Dimitroff, B. D.; Rawson, J. M.; Selleck, S. B. *Dev. Cell* **2004**, *7*, 513.
(67) Desbordes, S. C.; Sanson, B. *Development* **2003**, *130*, 6245.
(68) Kreuger, J.; Perez, L.; Giraldez, A. J.; Cohen, S. M. *Dev. Cell* **2004**, *7*, 503.
(69) Song, H. H.; Shi, W.; Xiang, Y. Y.; Filmus, J. *J. Biol. Chem.* **2005**, *280*, 2116.
(70) Iozzo, R. V.; Eichstetter, I.; Danielson, K. G. *Cancer Res.* **1995**, *55*, 3495.
(71) Clark, C. C.; Cohen, I.; Eichstetter, I.; Cannizzaro, L. A.; McPherson, J. D.; Wasmuth, J. J.; Iozzo, R. V. *Genomics* **1993**, *18*, 249.
(72) Galli, A.; Roure, A.; Zeller, R.; Dono, R. *Development* **2003**, *130*, 4919.
(73) Pacifici, M.; Shimo, T.; Gentili, C.; Kirsch, T.; Freeman, T. A.; Enomoto-Iwamoto, M.; Iwamoto, M.; Koyama, E. *J. Bone Miner. Metab.* **2005**, *23*, 191.

(74) Rawson, J. M.; Dimitroff, B.; Johnson, K. G.; Ge, X.; Van Vactor, D.; Selleck, S. B. *Curr. Biol.* **2005**, *15*, 833.
(75) Habuchi, H.; Habuchi, O.; Kimata, K. *Glycoconjugate J.* **2004**, *21*, 47.
(76) Holt, C. E.; Dickson, B. J. *Neuron* **2005**, *46*, 169.
(77) Bellaiche, Y.; The, I.; Perrimon, N. *Nature* **1998**, *394*, 85.
(78) The, I.; Bellaiche, Y.; Perrimon, N. *Mol. Cell* **1999**, *4*, 633.
(79) Toyoda, H.; Kinoshita-Toyoda, A.; Selleck, S. B. *J. Biol. Chem.* **2000**, *275*, 2269.
(80) Bornemann, D. J.; Duncan, J. E.; Staatz, W.; Selleck, S.; Warrior, R. *Development* **2004**, *131*, 1927.
(81) Lin, X.; Wei, G.; Shi, Z.; Dryer, L.; Esko, J. D.; Wells, D. E.; Matzuk, M. M. *Dev. Biol.* **2000**, *224*, 299.
(82) Inatani, M.; Irie, F.; Plump, A. S.; Tessier-Lavigne, M.; Yamaguchi, Y. *Science* **2003**, *302*, 1044.
(83) Koziel, L.; Kunath, M.; Kelly, O. G.; Vortkamp, A. *Dev. Cell* **2004**, *6*, 801.
(84) Lee, J. S.; von der Hardt, S.; Rusch, M. A.; Stringer, S. E.; Stickney, H. L.; Talbot, W. S.; Geisler, R.; Nüsslein-Volhard, C.; Selleck, S. B.; Chien, C. B.; Roehl, H. *Neuron* **2004**, *44*, 947.
(85) Siekmann, A. F.; Brand, M. *Dev. Dyn.* **2005**, *232*, 498.
(86) Hecht, J. T.; Hall, C. R.; Snuggs, M.; Hayes, E.; Haynes, R.; Cole, W. G. *Bone* **2002**, *31*, 199.
(87) Vortkamp, A.; Lee, K.; Lanske, B.; Segre, G. V.; Kronenberg, H. M.; Tabin, C. J. *Science* **1996**, *273*, 613.
(88) Lin, X.; Perrimon, N. *Nature* **1999**, *400*, 281.
(89) Lin, X.; Buff, E. M.; Perrimon, N.; Michelson, A. M. *Development* **1999**, *126*, 3715.
(90) Ringvall, M.; Ledin, J.; Holmborn, K.; van Kuppevelt, T.; Ellin, F.; Eriksson, I.; Olofsson, A. M.; Kjellén, L.; Forsberg, E. *J. Biol. Chem.* **2000**, *275*, 25926.
(91) Ledin, J.; Staatz, W.; Li, J. P.; Götte, M.; Selleck, S.; Kjellén, L.; Spillmann, D. *J. Biol. Chem.* **2004**, *279*, 42732.
(92) Forsberg, E.; Kjellén, L. *J. Clin. Invest.* **2001**, *108*, 175.
(93) Humphries, D. E.; Wong, G. W.; Friend, D. S.; Gurish, M. F.; Qiu, W. T.; Huang, C.; Sharpe, A. H.; Stevens, R. L. *Nature* **1999**, *400*, 769.
(94) Forsberg, E.; Pejler, G.; Ringvall, M.; Lunderius, C.; Tomasini-Johansson, B.; Kusche-Gullberg, M.; Eriksson, I.; Ledin, J.; Hellman, L.; Kjellén, L. *Nature* **1999**, *400*, 773.
(95) Samoszuk, M.; Corwin, M.; Yu, H.; Wang, J.; Nalcioglu, O.; Su, M. Y. *Thromb. Haemostasis* **2003**, *90*, 351.
(96) Bulow, H. E.; Hobert, O. *Neuron* **2004**, *41*.
(97) Kinnunen, T.; Huang, Z.; Townsend, J.; Gatdula, M. M.; Brown, J. R.; Esko, J. D.; Turnbull, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1507.
(98) Sen, J.; Goltz, J. S.; Stevens, L.; Stein, D. *Cell* **1998**, *95*, 471.
(99) Sergeev, P.; Streit, A.; Heller, A.; Steinmann-Zwicky, M. *Dev. Dyn.* **2001**, *220*, 122.
(100) Bullock, S. L.; Fletcher, J. M.; Beddington, R. S.; Wilson, V. A. *Genes Dev*. **1998**, *12*, 1894.
(101) Merry, C. L.; Bullock, S. L.; Swan, D. C.; Backen, A. C.; Lyon, M.; Beddington, R. S.; Wilson, V. A.; Gallagher, J. T. *J. Biol. Chem.* **2001**, *276*, 35429.
(102) Wilson, V. A.; Gallagher, J. T.; Merry, C. L. *Glycoconjugate J.* **2002**, *19*, 347.
(103) Nogami, K.; Suzuki, H.; Habuchi, H.; Ishiguro, N.; Iwata, H.; Kimata, K. *J. Biol. Chem.* **2004**, *279*, 8219.
(104) Bink, R. J.; Habuchi, H.; Lele, Z.; Dolk, E.; Joore, J.; Rauch, G.-J.; Geisler, R.; Wilson, S. W.; den Hertog, J.; Kimata, K.; Zivkovic, D. *J. Biol. Chem.* **2003**, *278*, 31118.
(105) Iida-Hasegawa, N.; Furuhata, A.; Hayatsu, H.; Murakami, A.; Fujiki, K.; Nakayasu, K.; Kanai, A. *Invest. Ophthalmol. Vision Sci.* **2003**, *44*, 3272.
(106) HajMohammadi, S.; Enjyoji, K.; Princivalle, M.; Christi, P.; Lech, M.; Beeler, D.; Rayburn, H.; Schwartz, J. J.; Barzegar, S.; de Agostini, A. I.; Post, M. J.; Rosenberg, R. D.; Shworak, N. W. *J. Clin. Invest.* **2003**, *111*, 989.
(107) Weitz, J. I. *J. Clin. Invest.* **2003**, *111*, 952.
(108) Koyama, T.; Parkinson, J. F.; Sie, P.; Bang, N. U.; Muller-Berghaus, G.; Preissner, K. T. *Eur. J. Biochem.* **1991**, *198*, 563.
(109) Parkinson, J. F.; Koyama, T.; Bang, N. U.; Preissner, K. T. *Adv. Exp. Med. Biol.* **1992**, *313*, 177.
(110) Conrad, H. E. *Heparin-Binding Proteins*; Academic Press: New York, 1998.
(111) Cardin, A. D.; Weintraub, H. J. *Arteriosclerosis* **1989**, *9*, 21.
(112) Humphries, D. E.; Nicodemus, C. F.; Schiller, V.; Stevens, R. L. *J. Biol. Chem.* **1992**, *267*, 13558.
(113) Capila, I.; Linhardt, R. J. *Angew. Chem., Int. Ed.* **2002**, *41*, 391.
(114) Rabenstein, D. L. *Nat. Prod. Rep.* **2002**, *19*, 312.
(115) Lipscombe, R. J.; Nakhoul, A. M.; Sanderson, C. J.; Coombe, D. R. *J. Leukocyte Biol.* **1998**, *63*, 342.
(116) Mummery, R. S.; Rider, C. C. *J. Immunol.* **2000**, *165*, 5671.
(117) Spillmann, D.; Witt, D.; Lindahl, U. *J. Biol. Chem.* **1998**, *273*, 15487.
(118) Salek-Ardakani, S.; Arrand, J. R.; Shaw, D.; Mackett, M. *Blood* **2000**, *96*, 1879.
(119) Sadir, R.; Baleux, F.; Grosdidier, A.; Imberty, A.; Lortat-Jacob, H. *J. Biol. Chem.* **2001**, *276*, 8288.
(120) Menart, V.; Fonda, I.; Kenig, M.; Porekar, V. G. *Ann. N. Y. Acad. Sci.* **2002**, *973*, 194.
(121) Kawashima, H.; Watanabe, N.; Hirose, M.; Sun, X.; Atarashi, K.; Kimura, T.; Shikata, K.; Matsuda, M.; Ogawa, D.; Heljasvaara, R.; Rehn, M.; Pihlajaniemi, T.; Miyasaka, M. *J. Biol. Chem.* **2003**, *278*, 13069.
(122) Stringer, S. E.; Gallagher, J. T. *J. Biol. Chem.* **1997**, *272*, 20508.
(123) Gupta, P.; Oegema, T. R., Jr.; Brazil, J. J.; Dudek, A. Z.; Slungaard, A.; Verfaillie, C. M. *Blood* **1998**, *92*, 4641.
(124) Ashikari-Hada, S.; Habuchi, H.; Kariya, Y.; Itoh, N.; Reddi, A. H.; Kimata, K. *J. Biol. Chem.* **2004**, *279*, 12346.
(125) Powell, A. K.; Fernig, D. G.; Turnbull, J. E. *J. Biol. Chem.* **2002**, *277*, 28554.
(126) Loo, B. M.; Kreuger, J.; Jalkanen, M.; Lindahl, U.; Salmivirta, M. *J. Biol. Chem.* **2001**, *276*, 16868.
(127) Wang, F.; Kan, M.; Yan, G.; Xu, J.; McKeehan, W. L. *J. Biol. Chem.* **1995**, *270*, 10231.
(128) Chang, Z.; Meyer, K.; Rapraeger, A. C.; Friedl, A. *FASEB J.* **2000**, *14*, 137.
(129) Ornitz, D. M.; Yayon, A.; Flanagan, J. G.; Svahn, C. M.; Levi, E.; Leder, P. *Mol. Cell. Biol.* **1992**, *12*, 240.
(130) Springer, B. A.; Pantoliano, M. W.; Barbera, F. A.; Gunyuzlu, P. L.; Thompson, L. D.; Herblin, W. F.; Rosenfeld, S. A.; Book, G. W. *J. Biol. Chem.* **1994**, *269*, 26879.
(131) Kan, M.; Wu, X.; Wang, F.; McKeehan, W. L. *J. Biol. Chem.* **1999**, *274*, 15947.
(132) Spivak-Kroizman, T.; Lemmon, M. A.; Dikic, I.; Ladbury, J. E.; Pinchasi, D.; Huang, J.; Jaye, M.; Crumley, G.; Schlessinger, J.; Lax, I. *Cell* **1994**, *79*, 1015.
(133) Venkataraman, G.; Shriver, Z.; Raman, R.; Sasisekharan, R. *Science* **1999**, *286*.
(134) Mach, H.; Volkin, D. B.; Burke, C. J.; Middaugh, C. R.; Linhardt, R. J.; Fromm, J. R.; Loganathan, D.; Mattsson, L. *Biochemistry* **1993**, *32*, 5480.
(135) Turnbull, J. E.; Fernig, D. G.; Ke, Y.; Wilkinson, M. C.; Gallagher, J. T. *J. Biol. Chem.* **1992**, *267*, 10337.
(136) Moy, F. J.; Safran, M.; Seddon, A. P.; Kitchen, D.; Bohlen, P.; Aviezer, D.; Yayon, A.; Powers, R. *Biochemistry* **1997**, *36*, 4782.
(137) Lundin, L.; Larsson, H.; Kreuger, J.; Kanda, S.; Lindahl, U.; Salmivirta, M.; Claesson-Welsh, L. *J. Biol. Chem.* **2000**, *275*, 24653.
(138) Guimond, S. E.; Turnbull, J. E. *Curr. Biol.* **1999**, *9*, 1343.
(139) Zhang, Z.; Coomans, C.; David, G. *J. Biol. Chem.* **2001**, *276*, 41921.
(140) Rubin, J. S.; Day, R. M.; Breckenridge, D.; Atabey, N.; Taylor, W. G.; Stahl, S. J.; Wingfield, P. T.; Kaufman, J. D.; Schwall, R.; Bottaro, D. P. *J. Biol. Chem.* **2001**, *276*, 32977.
(141) Raines, E. W.; Ross, R. *J. Cell Biol.* **1992**, *116*, 533.
(142) Feyzi, E.; Lustig, F.; Fager, G.; Spillmann, D.; Lindahl, U.; Salmivirta, M. *J. Biol. Chem.* **1997**, *272*, 5518.
(143) Higashiyama, S.; Abraham, J. A.; Klagsbrun, M. *J. Cell Biol.* **1993**, *122*, 933.
(144) Raab, G.; Klagsbrun, M. *Biochim. Biophys. Acta* **1997**, *1333*, 179.
(145) Pankonin, M. S.; Gallagher, J. T.; Loeb, J. A. *J. Biol. Chem.* **2005**, *280*, 383.
(146) Ruhrberg, C.; Gerhardt, H.; Golding, M.; Watson, R.; Ioannidou, S.; Fujisawa, H.; Betsholtz, C.; Shima, D. T. *Genes Dev.* **2002**, *16*, 2684.
(147) Lange, T.; Guttmann-Raviv, N.; Baruch, L.; Machluf, M.; Neufeld, G. *J. Biol. Chem.* **2003**, *278*, 17164.
(148) El-Sheikh, A.; Liu, C.; Huang, H.; Edgington, T. S. *Cancer Res.* **2002**, *62*, 7118.
(149) Irie, A.; Habuchi, H.; Kimata, K.; Sanai, Y. *Biochem. Biophys. Res. Commun.* **2003**, *308*, 858.
(150) Rahmoune, H.; Rudland, P. S.; Gallagher, J. T.; Fernig, D. G. *Biochemistry* **1998**, *37*, 6003.
(151) Lyon, M.; Deakin, J. A.; Rahmoune, H.; Fernig, D. G.; Nakamura, T.; Gallagher, J. T. *J. Biol. Chem.* **1998**, *273*, 271.
(152) Whitelock, J. M.; Murdoch, A. D.; Iozzo, R. V.; Underwood, P. A. *J. Biol. Chem.* **1996**, *271*, 10079.
(153) Knox, S.; Melrose, J.; Whitelock, J. *Proteomics* **2001**, *1*, 1534.
(154) Whitelock, J. M.; Graham, L. D.; Melrose, J.; Murdoch, A. D.; Iozzo, R. V.; Underwood, P. A. *Matrix Biol.* **1999**, *18*, 163.
(155) Knox, S.; Merry, C.; Stringer, S.; Melrose, J.; Whitelock, J. *J. Biol. Chem.* **2002**, *277*, 14657.
(156) Ferrara, N. *Am. J. Physiol. Cell Physiol.* **2002**, *280*, C1358.
(157) Neufeld, G.; Cohen, T.; Gengrinovitch, S.; Poltorak, Z. *FASEB J.* **1999**, *13*, 9.
(158) Gille, H.; Kowalski, J.; Li, B.; LeCouter, J.; Moffat, B.; Zioncheck, T.; Pelletier, N.; Ferrara, N. *J. Biol. Chem.* **2001**, *276*, 3222.
(159) Fong, G.; Rossant, J.; Gertsenstein, M.; Breitman, M. *Nature* **1995**, *376*, 66.
(160) Shalaby, F.; Rossant, J.; Yamaguchi, T.; Gertsenstein, M.; Wu, X.; Breitman, M.; Schuh, A. *Nature* **1995**, *376*, 62.
(161) Gitay-Goren, H.; Soker, S.; Vlodavsky, I.; Neufeld, G. *J. Biol. Chem.* **1992**, *267*, 6093.
(162) Gengrinovitch, S.; Berman, B.; David, G.; Witte, L.; Neufeld, G.; Ron, D. *J. Biol. Chem.* **1999**, *274*, 10816.

(163) Izvolsky, K. I.; Shoykhet, D.; Yang, Y.; Yu, Q.; Nugent, M. A.; Cardoso, W. V. *Dev. Biol.* **2003**, *258*, 185.
(164) Merry, C. L.; Wilson, V. A. *Biochim. Biophys. Acta* **2002**, *1573*, 319.
(165) van Barlingen, H. H.; Kleinveld, H. A.; Erkelens, D. W.; de Bruin, T. W. *Metabolism* **1997**, *46*, 650.
(166) Parthasarathy, N.; Goldberg, I. J.; Sivaram, P.; Mulloy, B.; Flory, D. M.; Wagner, W. D. *J. Biol. Chem.* **1994**, *269*, 22391.
(167) Futamura, M.; Dhanasekaran, P.; Handa, T.; Phillips, M. C.; Lund-Katz, S.; Saito, H. *J. Biol. Chem.* **2005**, *280*, 5414.
(168) Shriver, Z.; Liu, D.; Sasisekharan, R. *Trends Cardiovasc. Med.* **2002**, *12*, 71.
(169) Bourin, M. C.; Lindahl, U. *Biochem. J.* **1993**, *289*, 313.
(170) Petitou, M.; Hérault, J. P.; Bernat, A.; Driguez, P. A.; Duchaussoy, P.; Lormeau, J. C.; Herbert, J. M. *Nature* **1999**, *398*, 417.
(171) Roswit, W. T.; Halme, J.; Jeffrey, J. J. *Arch. Biochem. Biophys.* **1983**, *225*, 285.
(172) Isnard, N.; Robert, L.; Renard, G. *Cell Biol. Int.* **2003**, *27*, 779.
(173) Yu, W. H.; Woessner, J. F., Jr. *J. Biol. Chem.* **2000**, *275*, 4183.
(174) Kishnani, N. S.; Staskus, P. W.; Yang, T. T.; Masiarz, F. R.; Hawkes, S. P. *Matrix Biol.* **1995**, *14*, 479.
(175) Butler, G. S.; Apte, S. S.; Willenbrock, F.; Murphy, G. *J. Biol. Chem.* **1999**, *274*, 10846.
(176) Walker, A.; Gallagher, J. T. *Biochem. J.* **1996**, *317*, 871.
(177) Parthasarathy, N.; Gotow, L. F.; Bottoms, J. D.; Kute, T. E.; Wagner, W. D.; Mulloy, B. *J. Biol. Chem.* **1998**, *273*, 21111.
(178) Feitsma, K.; Hausser, H.; Robenek, H.; Kresse, H.; Vischer, P. *J. Biol. Chem.* **2000**, *275*, 9396.
(179) Vischer, P.; Feitsma, K.; Schon, P.; Volker, W. *Eur. J. Cell Biol.* **1997**, *73*, 332.
(180) Keller, K. M.; Keller, J. M.; Kuhn, K. *Biochim. Biophys. Acta* **1986**, *882*, 1.
(181) LeBaron, R. G.; Höök, A.; Esko, J. D.; Gay, S.; Höök, M. *J. Biol. Chem.* **1989**, *264*, 7950.
(182) Specks, U.; Mayer, U.; Nischt, R.; Spissinger, T.; Mann, K.; Timpl, R.; Engel, J.; Chu, M. L. *EMBO J.* **1992**, *11*, 4281.
(183) Tu, H.; Sasaki, T.; Snellman, A.; Gohring, W.; Pirila, P.; Timpl, R.; Pihlajaniemi, T. *J. Biol. Chem.* **2002**, *277*, 23092.
(184) Blackhall, F. H.; Merry, C. L.; Lyon, M.; Jayson, G. C.; Folkman, J.; Javaherian, K.; Gallagher, J. T. *Biochem. J.* **2003**, *375*, 131.
(185) Kreuger, J.; Matsumoto, T.; Vanwildemeersch, M.; Sasaki, T.; Timpl, R.; Claesson-Welsh, L.; Spillmann, D.; Lindahl, U. *EMBO J.* **2002**, *21*, 6303.
(186) Bengtsson, E.; Morgelin, M.; Sasaki, T.; Timpl, R.; Heinegard, D.; Aspberg, A. *J. Biol. Chem.* **2002**, *277*, 15061.
(187) Haugen, P. K.; Letourneau, P. C.; Drake, S. L.; Furcht, L. T.; McCarthy, J. B. *J. Neurosci.* **1992**, *12*, 2597.
(188) Drake, S. L.; Klein, D. J.; Mickelson, D. J.; Oegema, T. R.; Furcht, L. T.; McCarthy, J. B. *J. Cell Biol.* **1992**, *117*, 1331.
(189) Brauer, P. R.; Keller, K. M.; Keller, J. M. *Development* **1990**, *110*, 805.
(190) Riopelle, R. J.; Dow, K. E. *Brain Res.* **1990**, *525*, 92.
(191) van Bruggen, M. C.; Kramers, K.; Hylkema, M. N.; van den Born, J.; Bakker, M. A.; Assmann, K. J.; Smeenk, R. J.; Berden, J. H. *Am. J. Pathol.* **1995**, *146*, 753.
(192) Makino, H.; Ikeda, S.; Haramoto, T.; Ota, Z. *Nephron* **1992**, *61*, 415.
(193) Andriessen, M. P.; van den Born, J.; Latijnhouwers, M. A.; Bergers, M.; van de Kerkhof, P. C.; Schalkwijk, J. *J. Pathol.* **1997**, *183*, 264.
(194) Gupta, K.; Gupta, P.; Solovey, A.; Hebbel, R. P. *Biochim. Biophys. Acta* **1999**, *1453*, 63.
(195) Bruno, E.; Luikart, S. D.; Long, M. W.; Hoffman, R. *Exp. Hematol.* **1995**, *23*, 1212.
(196) Erdman, R.; Stahl, R. C.; Rothblum, K.; Chernousov, M. A.; Carey, D. J. *J. Biol. Chem.* **2002**, *277*, 7619.
(197) Rothblum, K.; Stahl, R. C.; Carey, D. J. *J. Biol. Chem.* **2004**, *279*, 51282.
(198) Takagaki, K.; Munakata, H.; Kakizaki, I.; Iwafune, M.; Itabashi, T.; Endo, M. *J. Biol. Chem.* **2002**, *277*, 8882.
(199) Turnbull, J. E.; Hopwood, J. J.; Gallagher, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 2698.
(200) Drummond, K. J.; Yates, E. A.; Turnbull, J. E. *Proteomics* **2001**, *1*, 304.
(201) Volpi, N.; Maccari, F. *Electrophoresis* **2002**, *23*, 4060.
(202) Volpi, N. *Anal. Biochem.* **1999**, *273*, 229.
(203) Wang, L.; Malsch, R.; Harenberg, J. *Semin. Thromb. Hemostasis* **1997**, *23*, 11.
(204) Turnbull, J. E. *Methods Mol. Biol.* **2001**, *171*, 141.
(205) Ii, T.; Kubota, M.; Okuda, S.; Hirano, T.; Ohashi, M. *Glycoconjugate J.* **1995**, *12*, 162.
(206) Duteil, S.; Gareil, P.; Girault, S.; Mallet, A.; Feve, C.; Siret, L. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1889.
(207) Pope, R. M.; Raska, C. S.; Thorp, S. C.; Liu, J. *Glycobiology* **2001**, *11*, 505.
(208) Juhasz, P.; Biemann, K. *Carbohydr. Res.* **1995**, *270*.
(209) Iwafune, M.; Kakizaki, I.; Nakazawa, H.; Nukatsuka, I.; Endo, M.; Takagaki, K. *Anal. Biochem.* **2004**, *325*.
(210) Thanawiroon, C.; Rice, K. G.; Toida, T.; Linhardt, R. J. *J. Biol. Chem.* **2004**, *279*, 2608.
(211) Kuberan, B.; Lech, M.; Zhang, L.; Wu, Z. L.; Beeler, D. L.; Rosenberg, R. D. *J. Am. Chem. Soc.* **2002**, *124*, 8707.
(212) Cooper, C. A.; Harrison, M. J.; Wilkins, M. R.; Packer, N. H. *Nucleic Acids Res.* **2001**, *29*, 332.
(213) Cooper, C. A.; Joshi, H. J.; Harrison, M. J.; Wilkins, M. R.; Packer, N. H. *Nucleic Acids Res.* **2003**, *31*, 511.
(214) Zak, B. M.; Crawford, B. E.; Esko, J. D. *Biochim. Biophys. Acta* **2002**, *1573*, 346.
(215) Vlodavsky, I.; Goldshmidt, O.; Zcharia, E.; Atzmon, R.; Rangini-Guatta, Z.; Elkin, M.; Peretz, T.; Friedmann, Y. *Semin. Cancer Biol.* **2002**, *12*, 121.
(216) Goldshmidt, O.; Zcharia, E.; Abramovitch, R.; Metzger, S.; Aingorn, H.; Friedmann, Y.; Schirrmacher, V.; Mitrani, E.; Vlodavsky, I. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10031.
(217) Sanderson, R. D.; Yang, Y.; Suva, L. J.; Kelly, T. *Matrix Biol.* **2004**, *23*, 341.
(218) Bernfield, M.; Gotte, M.; Park, P. W.; Reizes, O.; Fitzgerald, M. L.; Lincecum, J.; Zako, M. *Annu. Rev. Biochem.* **1999**, *68*, 729.
(219) Iozzo, R. V. *J. Clin. Invest.* **2001**, *108*, 165.
(220) Dunlevy, J. R.; Hassell, J. R. *Heparan sulfate proteoglycans in basement membranes: Perlecan, agrin and collagen XVIII*; Marcel Dekker: New York, 2000; p 275.
(221) Iozzo, R. V. *Annu. Rev. Biochem.* **1998**, *67*, 609.
(222) Yurchenco, P. D.; O'Rear, J. J. *Curr. Opin. Cell Biol.* **1994**, *6*, 674.
(223) Nugent, M. A.; Nugent, H. M.; Iozzo, R. V.; Sanchack, K.; Edelman, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6722.
(224) Noonan, D. M.; Fulle, A.; Valente, P.; Cai, S.; Horigan, E.; Sasaki, M.; Yamada, Y.; Hassell, J. R. *J. Biol. Chem.* **1991**, *266*, 22939.
(225) Kallunki, P.; Tryggvason, K. *J. Cell Biol.* **1992**, *116*, 559.
(226) Murdoch, A. D.; Dodge, G. R.; Cohen, I.; Tuan, R. S.; Iozzo, R. V. *J. Biol. Chem.* **1992**, *267*, 8544.
(227) Handler, M.; Yurchenco, P. D.; Iozzo, R. V. *Dev. Dyn.* **1997**, *210*, 130.
(228) Arikawa-Hirasawa, E.; Watanabe, H.; Takami, H.; Hassell, J. R.; Yamada, Y. *Nat. Genet.* **1999**, *23*, 354.
(229) Costell, M.; Gustafsson, E.; Aszodi, A.; Morgelin, M.; Bloch, W.; Hunziker, E.; Addicks, K.; Timpl, R.; Fassler, R. *J. Cell Biol.* **1999**, *147*, 1109.
(230) Costell, M.; Carmona, R.; Gustafsson, E.; Gonzalez-Iriarte, M.; Fassler, R.; Munoz-Chapuli, R. *Circ. Res.* **2002**, *91*, 158.
(231) Nugent, M. A.; Karnovsky, M. J.; Edelman, E. R. *Circ. Res.* **1993**, *73*, 1051.
(232) Weiser, M. C.; Grieshaber, N. A.; Schwartz, P. E.; Majack, R. A. *Mol. Biol. Cell* **1997**, *8*, 999.
(233) Kinsella, M. G.; Tran, P. K.; Weiser-Evans, M. C.; Reidy, M.; Majack, R. A.; Wight, T. N. *Arterioscler Thromb Vasc Biol* **2003**, *23*, 608.
(234) Carmeliet, P.; Jain, R. K. *Nature* **2000**, *407*, 249.
(235) Aviezer, D.; Hecht, D.; Safran, M.; Eisinger, M.; David, G.; Yayon, A. *Cell* **1994**, *79*, 1005.
(236) Iozzo, R. V. *J. Cell Biol.* **1984**, *99*, 403.
(237) Tapanadechopone, P.; Tumova, S.; Jiang, X.; Couchman, J. R. *Biochem. J.* **2001**, *355*, 517.
(238) Cohen, I. R.; Murdoch, A. D.; Naso, M. F.; Marchetti, D.; Berd, D.; Iozzo, R. V. *Cancer Res.* **1994**, *54*, 5771.
(239) Iozzo, R. V.; Cohen, I. R.; Grassel, S.; Murdoch, A. D. *Biochem. J.* **1994**, *302*, 625.
(240) Aviezer, D.; Iozzo, R. V.; Noonan, D. M.; Yayon, A. *Mol. Cell. Biol.* **1997**, *17*, 1983.
(241) Adatia, R.; Albini, A.; Carlone, S.; Giunciuglio, D.; Benelli, R.; Santi, L.; Noonan, D. M. *Ann Oncol* **1997**, *8*, 1257.
(242) Sharma, B.; Handler, M.; Eichstetter, I.; Whitelock, J. M.; Nugent, M. A.; Iozzo, R. V. *J. Clin. Invest.* **1998**, *102*.
(243) Mongiat, M.; Taylor, K.; Otto, J.; Aho, S.; Uitto, J.; Whitelock, J. M.; Iozzo, R. V. *J. Biol. Chem.* **2000**, *275*, 7095.
(244) Ghiselli, G.; Eichstetter, I.; Iozzo, R. V. *Biochem. J.* **2001**, *359*, 153.
(245) Timpl, R. *Experientia* **1993**, *49*, 417.
(246) SundarRaj, N.; Fite, D.; Ledbetter, S.; Chakravarti, S.; Hassell, J. R. *J. Cell Sci.* **1995**, *108*, 2663.
(247) Klein, G.; Conzelmann, S.; Beck, S.; Timpl, R.; Muller, C. A. *Matrix Biol.* **1995**, *14*, 457.
(248) Gauer, S.; Schulze-Lohoff, E.; Schleicher, E.; Sterzel, R. B. *Eur. J. Cell Biol.* **1996**, *70*, 233.
(249) Folkman, J. *Semin. Oncol.* **2002**, *29*, 15.
(250) Jain, R. K. *Nat. Med.* **2001**, *7*, 987.
(251) Folkman, J.; D'Amore, P. A. *Cell* **1996**, *87*, 1153.
(252) Mongiat, M.; Sweeney, S. M.; San Antonio, J. D.; Fu, J.; Iozzo, R. V. *J. Biol. Chem.* **2003**, *278*, 4238.
(253) Bix, G.; Fu, J.; Gonzalez, E. M.; Macro, L.; Barker, A.; Campbell, S.; Zutter, M. M.; Santoro, S. A.; Kim, J. K.; Höök, M.; Reed, C. C.; Iozzo, R. V. *J. Cell Biol.* **2004**, *166*, 97.
(254) Bix, G.; Iozzo, R. V. *Trends Cell Biol.* **2005**, *15*, 52.
(255) Filmus, J.; Song, H. H. *Glypicans*; Marcel Dekker Inc.: New York, 2000, p 161.
(256) Filmus, J.; Selleck, S. B. *J. Clin. Invest.* **2001**, *108*, 497.

(257) Kleeff, J.; Ishiwata, T.; Kumbasar, A.; Friess, H.; Buchler, M. W.; Lander, A. D.; Korc, M. *J. Clin. Invest.* **1998**, *102*, 1662.
(258) Vlodavsky, I.; Friedmann, Y.; Elkin, M.; Aingorn, H.; Atzmon, R.; Ishai-Michaeli, R.; Bitan, M.; Pappo, O.; Peretz, T.; Michal, I.; Spector, L.; Pecker, I. *Nat. Med.* **1999**, *5*, 793.
(259) Hulett, M. D.; Freeman, C.; Hamdorf, B. J.; Baker, R. T.; Harris, M. J.; Parish, C. R. *Nat. Med.* **1999**, *5*, 803.
(260) Zetser, A.; Levy-Adam, F.; Kaplan, V.; Gingis-Velitski, S.; Bashenko, Y.; Schubert, S.; Flugelman, M. Y.; Vlodavsky, I.; Ilan, N. *J. Cell Sci.* **2004**, *117*, 2249.
(261) Zcharia, E.; Metzger, S.; Chajek-Shaul, T.; Aingorn, H.; Elkin, M.; Friedmann, Y.; Weinstein, T.; Li, J. P.; Lindahl, U.; Vlodavsky, I. *FASEB J.* **2004**, *18*, 252.
(262) Gong, F.; Jemth, P.; Escobar Galvis, M. L.; Vlodavsky, I.; Horner, A.; Lindahl, U.; Li, J. P. *J. Biol. Chem.* **2003**, *278*, 35152.
(263) Zhou, Z.; Wang, J.; Cao, R.; Morita, H.; Soininen, R.; Chan, K. M.; Liu, B.; Cao, Y.; Tryggvason, K. *Cancer Res.* **2004**, *64*, 4699.
(264) van den Heuvel, L. P.; Westenend, P. J.; van den Born, J.; Assmann, K. J.; Knoers, N.; Monnens, L. A. *Nephrol Dial Transplant* **1995**, *10*, 2205.
(265) Meikle, P. J.; Fuller, M.; Hopwood, J. J. *Cell. Mol. Biol.* **2003**, *49*, 769.
(266) Parkinson-Lawrence, E.; Turner, C.; Hopwood, J.; Brooks, D. *Biochem. J.* **2005**, *386*, 395.
(267) Grabowski, G. A.; Hopkin, R. J. *Annu Rev Genomics Hum Genet* **2003**, *4*, 403.
(268) Garl, P. J.; Wenzlau, J. M.; Walker, H. A.; Whitelock, J. M.; Costell, M.; Weiser-Evans, M. C.; Nemenoff, R. A.; Stenmark, K. R. *Circ. Res.* **2004**, *94*, 175.
(269) Walker, H. A.; Whitelock, J. M.; Garl, P. J.; Nemenoff, R. A.; Stenmark, K. R.; Weiser-Evans, M. C. *Mol. Biol. Cell* **2003**, *14*, 1941.
(270) Edwards, I. J.; Wagner, J. D.; Vogl-Willis, C. A.; Litwak, K. N.; Cefalu, W. T. *Cardiovasc. Diabetol* **2004**, *3*, 6.
(271) Trybala, E.; Bergström, T.; Svennerholm, B.; Jeansson, S.; Glorioso, J. C.; Olofsson, S. *J. Gen. Virol.* **1994**, *75*, 743.
(272) Moon, A. F.; Edavettal, S. C.; Krahn, J. M.; Munoz, E. M.; Negishi, M.; Linhardt, R. J.; Liu, J.; Pedersen, L. C. *J. Biol. Chem.* **2004**, *279*, 45185.
(273) Feyzi, E.; Trybala, E.; Bergström, T.; Lindahl, U.; Spillmann, D. *J. Biol. Chem.* **1997**, *272*, 24850.
(274) Warner, R. G.; Hundt, C.; Weiss, S.; Turnbull, J. E. *J. Biol. Chem.* **2002**, *277*, 18421.
(275) Conde-Knape, K. *Diabetes Metab. Res. Rev.* **2001**, *17*, 412.
(276) Kjellen, L.; Bielefeld, D.; Hook, M. *Diabetes* **1983**, *32*.
(277) Snow, A. D.; Mar, H.; Nochlin, D.; Sekiguchi, R. T.; Kimata, K.; Koike, Y.; Wight, T. N. *Am. J. Pathol.* **1990**, *137*, 1253.
(278) van Horssen, J.; Wilhelmus, M. M.; Heljasvaara, R.; Pihlajaniemi, T.; Wesseling, P.; de Waal, R. M.; Verbeek, M. M. *Brain Pathol.* **2002**, *12*, 456.
(279) Verbeek, M. M.; Otte-Holler, I.; van den Born, J.; van den Heuvel, L. P.; David, G.; Wesseling, P.; de Waal, R. M. *Am. J. Pathol.* **1999**, *155*, 2115.
(280) Castillo, G. M.; Ngo, C.; Cummings, J.; Wight, T. N.; Snow, A. D. *J. Neurochem.* **1997**, *69*, 2452.
(281) Castillo, G. M.; Lukito, W.; Wight, T. N.; Snow, A. D. *J. Neurochem.* **1999**, *72*, 1681.
(282) Scholefield, Z.; Yates, E. A.; Wayne, G.; Amour, A.; McDowell, W.; Turnbull, J. E. *J. Cell Biol.* **2003**, *163*, 97.
(283) Coombe, D. R.; Kett, W. C. *Cell. Mol. Life Sci.* **2005**, *62*, 410.
(284) Greinacher, A.; Michels, I.; Mueller-Eckhardt, C. *Thromb. Haemostasis* **1992**, *67*, 545.
(285) Brieger, D.; Dawes, J. *Thromb. Haemostasis* **1997**, *77*, 317.
(286) Van Aken, H.; Bode, C.; Darius, H.; Diehm, C.; Encke, A.; Gulba, D. C.; Haas, S.; Hacke, W.; Puhl, W.; Quante, M.; Riess, H.; Scharf, R.; Schellong, S.; Schror, T.; Schulte, K. L.; Tebbe, U. *Clin. Appl. Thromb. Hemostasis* **2001**, *7*, 195.
(287) McCart, G. M.; Kayser, S. R. *Ann. Pharmacother.* **2002**, *36*, 1042.
(288) Weitz, J. I.; Crowther, M. A. *Am. J. Cardiovasc. Drugs* **2003**, *3*, 201.
(289) Ragazzi, M.; Ferro, D. R.; Perly, B.; Torri, G.; Casu, B.; Sinay, P.; Petitou, M.; Choay, J. *Carbohydr. Res.* **1987**, *165*, 1.
(290) Petitou, M.; Lormeau, J. C.; Choay, J. *Semin. Thromb. Hemostasis* **1991**, *17*, 143.
(291) Turpie, A. G.; Eriksson, B. I.; Lassen, M. R.; Bauer, K. A. *Curr. Opin. Hematol.* **2003**, *10*, 327.
(292) Turpie, A. G.; Eriksson, B. I.; Bauer, K. A.; Lassen, M. R. *Chest* **2003**, *124*, 371S.
(293) Bauer, K. A. *Chest* **2003**, *124*, 364S.
(294) Jaurand, G.; Tabeur, C.; Petitou, M. *Carbohydr. Res.* **1994**, *255*, 295.
(295) Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Litjens, R. E.; Palmacci, E. R.; Seeberger, P. H. *Chemistry* **2003**, *9*, 140.
(296) Lohman, G. J.; Hunt, D. K.; Högermeier, J. A.; Seeberger, P. H. *J. Org. Chem.* **2003**, *68*, 7559.
(297) Disney, M. D.; Seeberger, P. H. *Chem. Biol.* **2004**, *11*, 1701.
(298) Ratner, D. M.; Adams, E. W.; Disney, M. D.; Seeberger, P. H. *ChemBioChem* **2004**, *5*, 1375.
(299) de Raucourt, E.; Mauray, S.; Chaubet, F.; Maiga-Revel, O.; Jozefowicz, M.; Fischer, A. M. *J. Biomed. Mater. Res.* **1998**, *41*, 49.
(300) Akashi, M.; Sakamoto, N.; Suzuki, K.; Kishida, A. *Bioconjugate Chem.* **1996**, *7*, 393.
(301) Sie, P.; Ofosu, F.; Fernandez, F.; Buchanan, M. R.; Petitou, M.; Boneu, B. *Br. J. Haematol.* **1986**, *64*, 707.
(302) Baba, M.; Nakajima, M.; Schols, D.; Pauwels, R.; Balzarini, J.; De Clercq, E. *Antiviral Res.* **1988**, *9*, 335.
(303) Ghosh, P.; Smith, M. *Biogerontology* **2002**, *3*, 85.
(304) Smith, M. M.; Ghosh, P.; Numata, Y.; Bansal, M. K. *Arthritis Rheum.* **1994**, *37*, 125.
(305) Yu, G.; Gunay, N. S.; Linhardt, R. J.; Toida, T.; Fareed, J.; Hoppensteadt, D. A.; Shadid, H.; Ferro, V.; Li, C.; Fewings, K.; Palermo, M. C.; Podger, D. *Eur. J. Med. Chem.* **2002**, *37*, 783.
(306) Cochran, S.; Li, C.; Fairweather, J. K.; Kett, W. C.; Coombe, D. R.; Ferro, V. *J. Med. Chem.* **2003**, *46*, 4601.
(307) Parish, C. R.; Freeman, C.; Hulett, M. D. *Biochim. Biophys. Acta* **2001**, *1471*, M99.
(308) Khachigian, L. M.; Parish, C. R. *Cardiovasc. Drug Rev.* **2004**, *22*, 1.
(309) Freeman, C.; Liu, L.; Banwell, M. G.; Brown, K. J.; Bezos, A.; Ferro, V.; Parish, C. R. *J. Biol. Chem.* **2005**, *280*, 8842.
(310) Ashikari-Hada, S.; Habuchi, H.; Kariya, Y.; Itoh, N.; Reddi, A. H.; Kimata, K. *J. Biol. Chem.* **2004**, *279*.
(311) van de Westerlo, E. M.; Smetsers, T. F.; Dennissen, M. A.; Linhardt, R. J.; Veerkamp, J. H.; van Muijen, G. N.; van Kuppevelt, T. H. *Blood* **2002**, *99*, 2427.
(312) Smits, N. C.; Robbesom, A. A.; Versteeg, E. M.; van de Westerlo, E. M.; Dekhuijzen, P. N.; van Kuppevelt, T. H. *Am. J. Respir. Cell Mol. Biol.* **2004**, *30*, 166.

CR010213M