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Heparan sulfate proteoglycans: structure, protein interactions and cell signaling

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ABSTRACT

Heparan sulfate proteoglycans are ubiquitously found at the cell surface and extracellular matrix in all the animal species. This review will focus on the structural characteristics of the heparan sulfate proteoglycans related to protein interactions leading to cell signaling. The heparan sulfate chains due to their vast structural diversity are able to bind and interact with a wide variety of proteins, such as growth factors, chemokines, morphogens, extracellular matrix components, enzymes, among others. There is a specificity directing the interactions of heparan sulfates and target proteins, regarding both the fine structure of the polysaccharide chain as well precise protein motifs. Heparan sulfates play a role in cellular signaling either as receptor or co-receptor for different ligands, and the activation of downstream pathways is related to phosphorylation of different cytosolic proteins either directly or involving cytoskeleton interactions leading to gene regulation. The role of the heparan sulfate proteoglycans in cellular signaling and endocytic uptake pathways is also discussed.

Key words: glycosaminoglycans and protein interactions, growth factors, focal adhesion, extracellular matrix, cell cycle, cell proliferation.

STRUCTURAL FEATURES OF HEPARAN SULFATE PROTEOGLYCANS

The most distinguishing features between heparin and heparan sulfate (HS) are their cellular localization, their occurrence in the animal kingdom, and thus their biological functions. Heparin and heparan sulfate are attached to different core proteins and found in different cellular compartments. Heparin is found exclusively inside storage vesicles of mast cells of some animal species (Nader et al. 1999a, 1980, Straus et al. 1982) whereas heparan sulfates are ubiquitous to the cell surface of both vertebrate and invertebrate species (Cassaro and Dietrich 1977, Dietrich et al. 1980, 1977, Nader et al. 1984).

Heparin and heparan sulfates are polydisperse linear polymers that share structural similarities. They are composed of alternate units of α-D-glucosamine (GlcN) and uronic acid, either β-D-glucuronic acid (GlcA) or α-L-iduronic acid (IdoA), joined together by (1$\rightarrow$ 4) glycosidic linkages. In heparan sulfate the GlcN can be either N-sulfated or N-acetylated, whereas in heparin the N-acetyl groups correspond to less then 5%. Furthermore, heparin shows higher degree of sulfation (2.3–2.8 sulfates/disaccharide) when compared to heparan sulfates (0.6–1.5 sulfates/disaccharide).

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The sequences of the different types of disaccharides in heparan sulfate and heparin were established using chemical, enzymatic and NMR analyses of the intact polymers and their fragments. Figure 1A summarizes the sites of action of the chemical and enzymatic protocols.

Chemical procedures, such as nitrous acid degradation of the polymer at different pHs, can furnish important data on the structure of these compounds. At low pH and room temperature, the N-sulfated GlcNs in the heparan sulfate are susceptible to degradation yielding fragments with ranges of molecular weights that depend on the distributions of the N-sulfated GlcN residues in the chain and bearing an anydromannose at the reducing terminal end of the fragment. Thus, the obtained fragments will contain clusters of N-acetylated GlcN, since N-acetylated amino sugars are not affected (Conrad 2001). On the other hand, hydrazinolysis coupled with nitrous acid treatment at pH 4.0 affects the N-acetylated portion of the polymer.

Bacterial glycosaminoglycan lyases and the animal endo-hydrolases described so far have also been used to ascertain the disaccharide sequences in heparan sulfate chains. Mollusk endo-β-glucuronidase and α-D-N-acetylglucosaminidase degrade heparan sulfates chains yielding oligosaccharides enriched in O-sulfates and IdoA residues. Furthermore, heparan sulfate can be degraded by a class of mammalian endo-hydrolases known as heparanases, which are endo-β-glucuronidases that cleave β-D-glucuronyl (1$\rightarrow$4) D-GlcN N-sulfated located after a disaccharide composed of α-L-iduronyl (1$\rightarrow$4) D-GlcN N-acetylated. Figure 1A summarizes the sites of action of the chemical and enzymatic procedures.

Characteristic ^{1}H and ^{13}C chemical shifts have been identified for the individual residues, and the relative abundance of these moieties can be quantitatively determined by integrating the proton signals. By a combination of two-dimensional NMR techniques such as correlated spectroscopy (COSY), nuclear overhauser effect (NOESY) and total correlation spectroscopy (TOCSY) for ^{1}H, and heteronuclear single-quantum coherence (HSQC) for ^{13}C, some of the sequences can be determined (Chavante et al. 2000, Chuang et al. 2001, Dietrich et al. 1999, Ferreira et al. 1993, Guerrini et al. 2001, 2002, Nader et al. 1999b, 1990).

The combined used of these approaches made it possible to establish the sequence of characteristic domains in the structure of heparan sulfates from different origins (Fig. 1B). Thus heparan sulfates from both vertebrate and invertebrate tissues contain common structural features such as N-acetylated and N-sulfated GlcN, domains consisting only of GlcA-containing disaccharides with no 6-O-sulfate substitutions (susceptible to heparitinase I, endo-β-glucuronidases, and nitrous acid pH 4.0) and a more sulfated region consisting of IdoA-containing disaccharides (susceptible to heparitinase II and nitrous acid pH 1.5). At the non-reducing end all polymers contain GlcN N-sulfate or GlcN N,6-disulfate followed by a disaccharide composed of IdoA 2-O-sulfated linked to GlcN 2,6-disulfated (susceptible to heparinase). A peculiar tetrasaccharide is positioned between the two regions and was identified in all heparan sulfates. The N-acetylated GlcA domain is close to the protein core and contains the reducing terminal of the chain (Dietrich et al. 1983, 1998, Ferreira et al. 1993, Nader et al. 1987, 1999b, Tersariol et al. 1994).

Heparan sulfates are absent in protista, plantae and fungi, and their appearance in the animal kingdom coincides with the emergence of eumetazoa, which are animals that display true tissues, being ubiquitously found in all tissues and species analyzed (Cassaro and Dietrich 1977, Dietrich et al. 1980, 1977, Gomes and Dietrich 1982, Nader et al. 1984, Toledo and Dietrich 1977). Non-sulfated version of heparan sulfate, named heparosan, is found in the capsules of some pathogenic bacteria, thus acting as molecular camouflages protecting the microbe and enhancing infection (DeAngelis 2002).

Heparan sulfates are attached to different core proteins and found at the cellular surface and extracellular matrices, such as basal membrane. The chains at the cell surface can be attached to transmembrane proteins as in syndecans or through a glycosylphosphatidylinositol-anchored core protein, as in glypicans (Bernfield et al. 1999, Fransson 2003, Fransson et al. 2004, Tantravahi et al. 1986, Tkachenko et al. 2005). Table I shows different proteins that can bear heparan sulfate chains and their cellular localization.

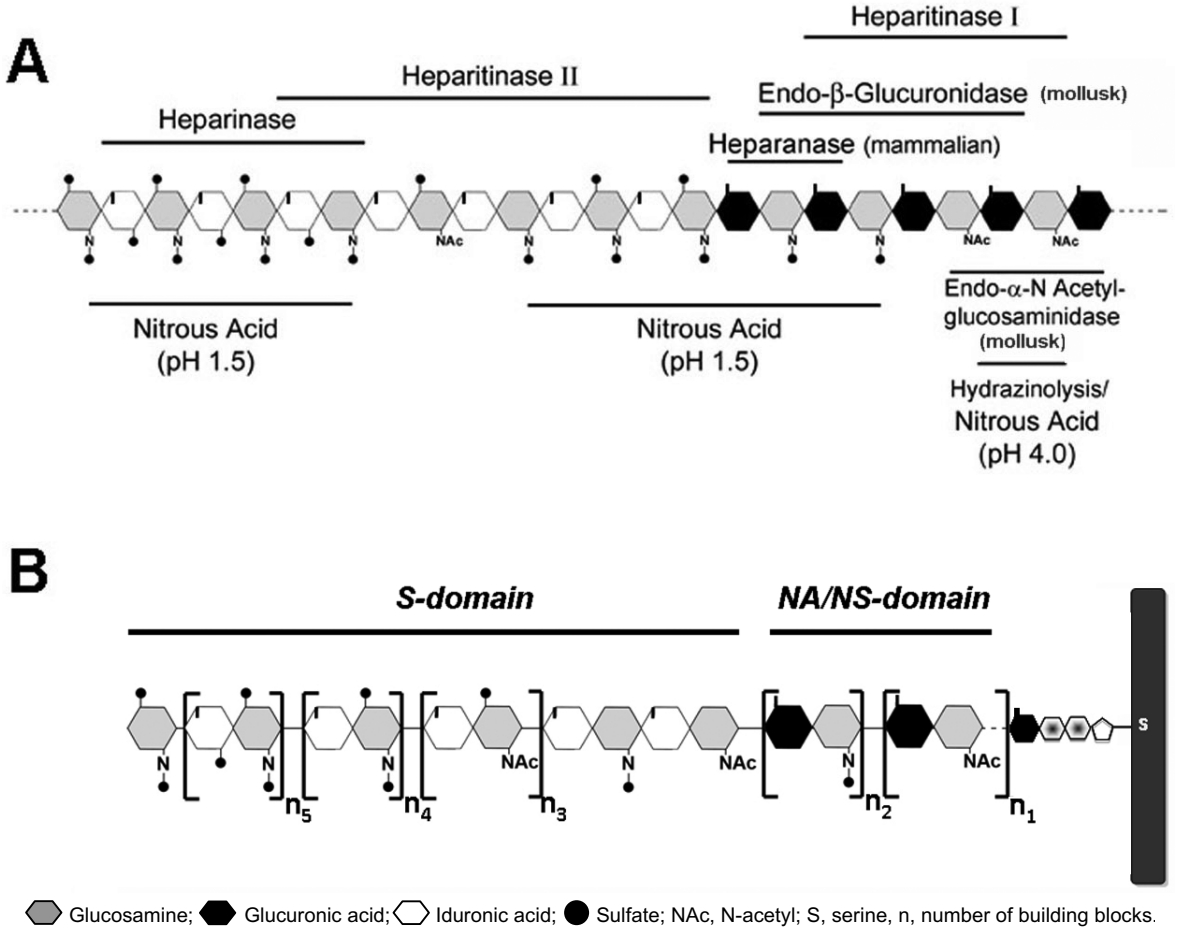

Fig. 1 – Heparan Sulfate Proteoglycan Structural Characteristics. (A) Hypothetical heparin/heparan sulfate chain and site of action of different enzymatic and chemical depolymerization procedures. (B) Proposed structure for heparan sulfate from different origins. S-domain represents iduronic acid containing disaccharides and NA/NS-domain represents glucuronic acid containing disaccharides bearing glucosamine N-sulfate or N-acetylglucosamine.

HEPARAN SULFATES AND PROTEIN INTERACTIONS

Several works in the literature clearly show that there is a specificity directing the interactions of heparan sulfates and target proteins, regarding both the fine structure of the polysaccharide chain and precise protein motifs. Thus, they can interact with a diverse range of proteins leading to biological activities (Fig. 2). The heparan sulfate chains due to their vast structural diversity are able to bind and interact with a wide variety of proteins, such as growth factors, chemokines, morphogens, extracellular matrix components, and enzymes, among others. Table II lists some of the heparan sulfate binding proteins that modulate different biological processes through this interaction.

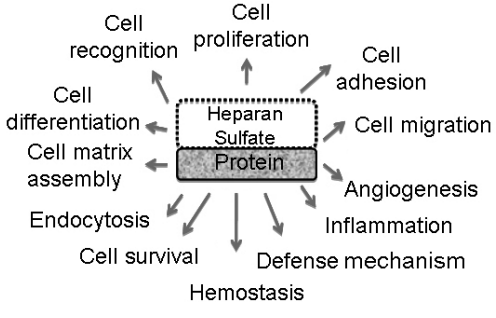

Fig. 2 – Biological activities modulated by the interaction of proteins with heparan sulfate.

These proteins contain relatively large numbers of the basic amino acids (lysine, arginine and in some cases histidine). These basic residues can be found in linear

TABLE I
Heparan sulfate proteoglycans.

Cell surface	References
Syndecan family (transmembrane)	
Syndecan 1	(Sanderson and Yang 2008)
Syndecan 2	(Oh and Couchman 2004)
Syndecan 3	(Bellin et al. 2002)
Syndecan 4	(Oh and Couchman 2004)
Glypican family	
(bound to the membrane by a	
glycosylphosphatidylinositol anchor)	(Filmus et al. 2008)
Glypican 1	(Fransson et al. 2004)
Glypican 2	(Filmus 2002)
Glypican 3	(Stigliano et al. 2009)
Glypican 4	(Huber et al. 1998)
Glypican 5	(Veugelers et al. 1997)
Glypican 6	(Veugelers et al. 1999)
CD44 (transmembrane)	(Henke et al. 1996)
Betaglycan	(Miyazono 1997)
Extracellular matrix	References
Perlecan	(Farach-Carson and Carson 2007)
Agrin	(Bezakova and Ruegg 2003)
Type XVIII collagen	(Iozzo 2005)
Testican family	
Testican 1	(Alliel et al. 1993)
Testican 2	(Schnepp et al. 2005)
Testican 3	(Nakada et al. 2003)

arrangements or in spatial folded clusters. Cardin and Weintraub proposed two consensus motifs, XBBXBX or XBBBXXBX, where B represents basic amino acids and X, hydropathic (neutral or hydrophobic) residue (Cardin and Weintraub 1989). Nevertheless, binding can also involve basic amino acids that are distant in linear sequence of the protein and that are brought together in the protein folded state (Capila and Linhardt 2002, Hileman et al. 1998, Krilleke et al. 2007, Mulloy and Linhardt 2001, Vives et al. 2004).

Considering the heparan sulfates, the specificity seems to be related to the distribution and conformation of β-D-GlcA and α-L-IdoA residues, relative amounts of N-acetyl or N-sulfate groups in the GlcN moiety, as well as the relative amounts and the position of O-sulfation of the uronic acid and GlcN units. Specific sequences of disaccharides can favor the interaction of the molecule with certain proteins and not to others. Up to now, besides specific sugar sequences bearing IdoA and enriched in sulfate groups (S-domain), it has been postulated that the conformational flexibility of the α-L-IdoA residue plays a pivotal role in protein interactions. IdoA residues can assume both $^1C_4$ chair and the $^2S_0$ skew boat conformation, thus allowing appropriate electrostatic interactions with basic amino acids on the protein (Casu et al. 1986, Ferro et al. 1990, Gallagher 2006, Habuchi et al. 2004, Mulloy 2005, Mulloy and Forster 2000, Noti and Seeberger 2005, Ragazzi et al. 1993). It has been shown that a heparin-derived tetrasaccharide that interacts with annexin V shows IdoA on the $^2S_0$ conformation, while the non-interacting tetrasaccharide the $^1C_4$ conformation (Capila et al. 2001, 1999, Ishitsuka et al. 1998). More recently, it has been suggested the N-acetylated region (NA-domain), which is rich in β-D-GlcA residues, also displays structural plasticity and hence could mediate protein interactions (Mobli et al. 2008).

TABLE II
Heparan sulfate binding proteins*.

Cell surface	References
L-selectin and P-selectin	(Ma and Geng 2000)
N-CAM (Neural Cell Adhesion Molecule)	(Cole et al. 1986)
PECAM-1 (Platelet Endothelial Cell Adhesion Molecule)	(Watt et al. 1993)
FGF receptor	(Powell et al. 2004)
HIP (Heparin/Heparan Sulfate Interaction Protein)	(Rohde et al. 1998)
MAC-1 (Monocyte Adhesion Molecule)	(Coombe et al. 1994)
Extracellular matrix	**References**
Collagens	(Sasisekharan et al. 2002)
Fibronectin	(Capila and Linhardt 2002)
HB-GAM (Heparin Binding Growth Associated Molecule)	(Taylor and Gallo 2006)
Laminin	(Utani et al. 2001)
Tenascin	(Saito et al. 2007)
Thrombospondin I and II	(Nunes et al. 2008)
Vitronectin	(Wilkins-Port and McKeown-Longo 1996)
Growth factors	**References**
HB-EGF family (Heparin Binding – Epidermal Growth Factors)	(Aviezer and Yayon 1994)
FGF family (Fibroblast Growth Factors)	(Gambarini et al. 1993)
VEGF (Vascular Endothelial Growth Factor)	(Iozzo and San Antonio 2001)
HDGF (Hepatoma Derived Growth Factor)	(Dietz et al. 2002)
PlGF (Placenta Growth Factor)	(Athanassiades and Lala 1998)
PDGF (Platelet-Derived Growth Factor)	(Sasisekharan et al. 2002)
TGF-β (Transforming Growth Factor-β)	(Sasisekharan et al. 2002)
HGF (Hepatocyte Growth Factor)	(Derksen et al. 2002)
Cytokines/Chemokines/Morphogens	**References**
BMP (bone morphogenetic protein)	(Hacker et al. 2005)
IL-1, -2, -3, -4, -5, -7, -8, -10, -12 (Interleukin)	(Koopmann et al. 1999)
IP-10 (Interferon -γ inducible protein 10)	(Handel et al. 2005)
CCL-2 (CC-chemokine ligand)	(Johnson et al. 2005)
GM-CSF (Granylocyte Macrophage Colony Stimulating Factor)	(Raman et al. 2005)
MCP-1, MCP-4 (Monocyte Chemoatractant Protein)	(Johnson et al. 2005)
RANTES (Regulated on Activation Normal T cell Expressed and Secreted)	(Johnson et al. 2005)
TNF-α (Tumor Necrosis Factor)	(Handel et al. 2005)
MIP-1 (Macrophage Inflammatory Protein)	(Vlodavsky et al. 2002)
PF-4 (Platelet factor 4)	(Sulpice et al. 2002)
Hh (Sonic Hedgehog)	(Hacker et al. 2005)
Wnt (Wingless wg)	(Hacker et al. 2005)
Others	**References**
DNA and RNA polymerases	(Furukawa and Bhavanandan 1983)
Superoxide dismutase	(Nozik-Grayck et al. 2005)
Angiogenin	(Soncin et al. 1997)
Cathepsins B and G	(Almeida et al. 2001)
Neutrophil elastase	(Campbell and Owen 2007)
Annexin V	(Mulloy and Linhardt 2001)
Prion	(Ben-Zaken et al. 2003)
β-amyloid protein	(Patey et al. 2008)
Na^+/Ca^{2+} exchanger protein	(Shinjo et al. 2002)
Myosin ATPase	(Tersariol et al. 1992)

Using heparin derived oligosaccharides and chemically modified molecules, the role of N-sulfated and N-acetylated domains, as well as the position of the O-sulfates, and the conformation of the uronic acid residue were investigated regarding the binding specificity to different proteins.

It is well established that fibroblast growth factors and their receptors are dependent on binding to heparan sulfate, and this interaction is an absolute requirement for full signaling. However most of the studies regarding the minimum structural features of the polysaccharide needed for the binding were concluded using chemico-enzymatically heparin derivatives as heparan sulfate analogs. Even though these heparin derivatives can be produced in high amounts, and thus can be used to elucidate some of the binding characteristics, they do not substitute the high diversity and thus the specificity that is found in the heparan sulfate polymers (Belford et al. 1992, Harmer 2006, Ishihara et al. 1993, Mohammadi et al. 2005a, Presta et al. 2005, Yates et al. 2004).

However, heparan sulfates show large sequences of GlcA linked to N-acetylated GlcN which are not present in heparins. Also, heparan sulfates show lower degree of sulfation, even in the IdoA residue, requisites that are described as important for the protein binding. So, this raises questions on how the binding could be affected by these domains in the heparan sulfate chains, which are the postulated polysaccharide for most of these biological interactions.

The structural requirements involved in the binding vary for each protein. Some important sequences for specific heparin/heparan sulfate-protein interactions, which are dependent mostly on the presence of IdoA, as well as the sulfation of the IdoA and the N-sulfation of the GlcN moiety, have been established (Jastrebova et al. 2006, Patel et al. 2008, Sampaio et al. 2006, Sasisekharan et al. 2002, Yates et al. 2004, Zhang et al. 2007). Furthermore, the protein interaction depends on the size of the chain, and the minimum fragment varies from a tetrasaccharide described for annexin V up to a dodecasaccharide for the FGF-2 receptor.

The sulfation pattern is another important requisite for the binding of heparin/heparan sulfate to proteins. For example, the growth factor PDGF-A is dependent mostly on the amounts of 2-O-sulfate in the IdoA residues (Feyzi et al. 1997). On the other hand, for the chemokine CCL-2, both 2-O-sulfate in the IdoA and N-sulfation of the GlcN are required (Crown et al. 2006). The interaction of heparan sulfate with FGF-4 receptor depends more on the number of 6-O-sulfate groups than on their precise location (Loo et al. 2001), and for FGF-receptor 2 the minimum structure for binding is an octasaccharide containing 2-O- and 6-O-sulfates and for signaling a dodecasaccharide (Walker et al. 1994). In a recent paper, using embryonic fibroblasts derived from knock-out mice for heparan sulfate 6-O-sulfotransferases 1- and 2, it was shown an important role of 6-O-sulfation patterns in FGF signaling (Sugaya et al. 2008). The binding of neuregulin-1 to erbB receptor depends mainly on the N-sulfate groups of heparan sulfate, followed by 2-O- and 6-O-sulfate groups (Pankonin et al. 2005). Interaction of endostatin to endothelial heparan sulfate shows differential requirements for specific sulfate groups where 6-O-sulfates play a dominant role in selectivity (Blackhall et al. 2003). Recently, it has been shown that 6-O-sulfation of heparan sulfate differentially regulates various fibroblast growth factor-dependent signalings in culture (Sugaya et al. 2008).

Specific structural features of heparan sulfate involved in protein interactions were illustrated by experiments using FGF-1 and heparan sulfates from various sources that exhibit different disaccharides assembling. The FGF-1 mitogenic activity varies among the different heparan sulfates. The oligosaccharide derived from a heparan sulfate containing only the GlcA domain with no 6-O-sulfation (NA/NS-domain) displays no activity, whereas the counterpart enriched in IdoA and 6-O-sulfation (S-domain) shows around 10 times the activity of the intact polymer (Fig. 1B). Furthermore, heparan sulfate purified from 3T3 fibroblasts has an effect about 100 times higher. These results indicate that endogenous heparan sulfate is the best elicitor for the FGF-1 mitogenic activity, and that the S-domain represents the FGF-1 binding site, indicating a highly specific interaction (Gambarini et al. 1993).

Thus, it appears that the specificity of the interactions heparan sulfate-protein depends on the overall organization of the glycosaminoglycan chain rather than on

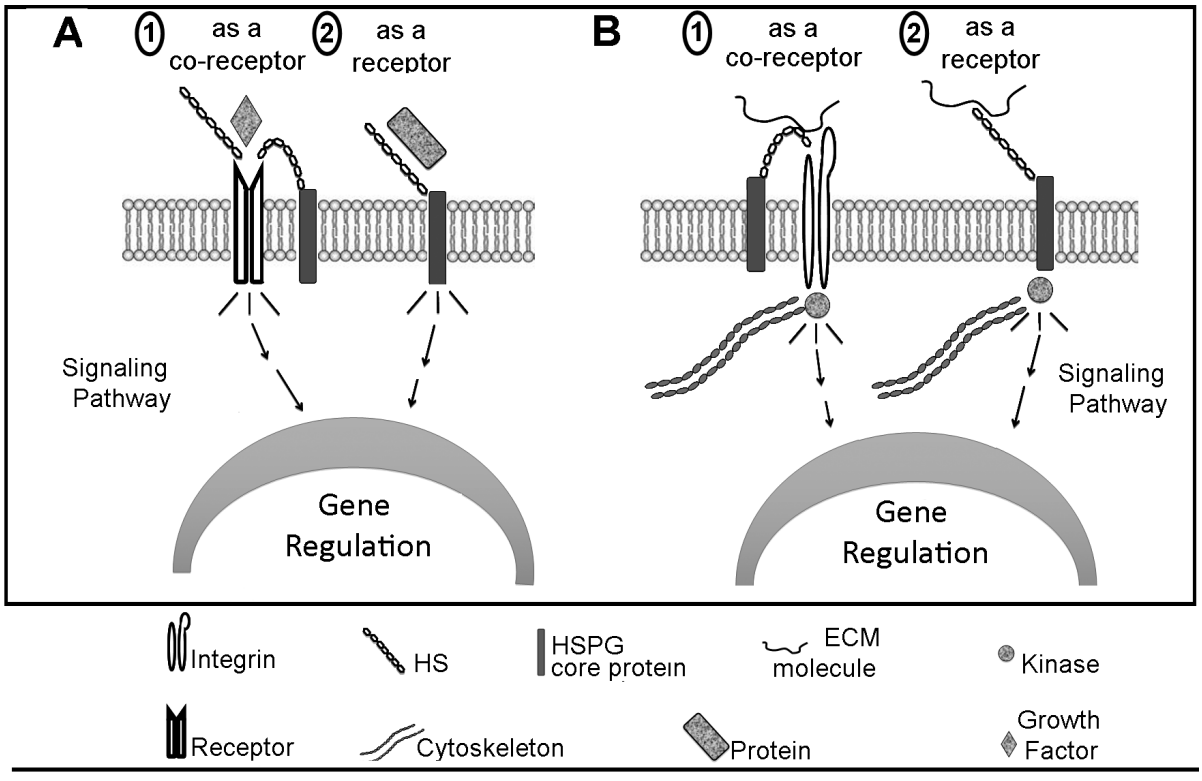

Fig. 3 – Heparan sufate proteoglycans features in cell signaling. HSPG can trigger cell response through signal transduction pathways as a receptor or co-receptor in a cytoskeleton independent (A) or dependent manner (B).

the fine structure of the individual sequences to achieve its functional role (Gambarini et al. 1993, Kreuger et al. 2006, Sampaio et al. 2006, Suarez et al. 2007).

HEPARAN SULFATE PROTEOGLYCANS AND CELL SIGNALING PATHWAYS

Heparan sulfate chains are located facing the extracellular compartment, and thus their biological roles can be related to assemble the extracellular matrices (Baeg et al. 2001, Iozzo 2005, Peretti et al. 2008), to modulate the activity of enzymes and/or their inhibitors (Almeida et al. 2001, Hausser et al. 2004, Nascimento et al. 2005, Raman et al. 2005, Sasaki et al. 1999, Whitelock et al. 1996, Yu and Woessner 2000, Yu et al. 2000), to provide an extracellular gradient of growth factors and chemokines (Ashikari-Hada et al. 2005, Grunert et al. 2008, Hacker et al. 2005, Kirkpatrick and Selleck 2007, Ng et al. 2006, Nugent and Iozzo 2000, Ruhrberg et al. 2002), and to prevent degradation of growth factors (Saksela et al. 1988), among others. Nevertheless, heparan sulfate proteoglycans can trigger cell response through signal transduction pathways, as well as by translocation to intracellular compartments, due to interactions of the polysaccharide chains and/or the core protein with specific ligands.

Heparan sulfates play a role in cellular signaling either as receptor or co-receptor for different ligands (Fig. 3). The activation of downstream pathways is related to phosphorylation of different cytosolic proteins either directly (Fig. 3A) or involving cytoskeleton interactions (Fig. 3B) leading to gene regulation.

In early 1990's Yayon and co-workers, using CHO cells defected in glycosaminoglycan biosynthesis, suggested heparan sulfates as low affinity receptors required for binding of FGF-2 to the high affinity site (Yayon et al. 1991). Such an obligatory interaction of low and high affinity FGF receptors suggested a novel mechanism for the regulation of growth factor-receptor interactions. Indeed, this effect of heparan sulfate is responsible for FGF receptor dimerization and activation, leading to cellular responses (Rapraeger et al. 1991, Spivak-Kroizman et al. 1994).

As described above, HSPGs can function as low-affinity receptors required for the activation of growth factor high-affinity receptor, which has tyrosine kinase activity. A substantial body of literature supports the concept of the ternary complex involving HSPG, growth factor and its high-affinity receptor (Fig. 3A-2).

Heparan sulfates as co-receptors for fibroblast growth factors with tyrosine kinase activity have been extensively studied and corroborated in other systems (Czubayko et al. 1997, Duchesne et al. 2006, Mohammadi et al. 2005a, b, Mongiat et al. 2000, Padera et al. 1999, Pellegrini et al. 2000, Powers et al. 2000, Rapraeger et al. 1994, Wiedlocha and Sorensen 2004, Wu et al. 1991). This general model has been also extended to many other growth factors, such as vascular endothelial growth factor (Ashikari-Hada et al. 2005, Gitay-Goren et al. 1992, Iozzo and San Antonio 2001, Stringer 2006), hepatocyte growth factor (Kemp et al. 2006, Rubin et al. 2001, Schwall et al. 1996), platelet-derived growth factor (Abramsson et al. 2007, Rolny et al. 2002), placenta growth factor (Athanassiades and Lala 1998), and heparin binding-epidermal growth factor (Aviezer and Yayon 1994).

Heparan sulfate proteoglycans can also interfere with serine/threonine kinase receptors, such as transforming growth factor-β and bone morphogenetic protein (Chen et al. 2006, Cohen 2003, Grunert et al. 2008, Rider 2006, Sasaki et al. 2008), tyrosine phosphatase receptors (Aricescu et al. 2002, Fox and Zinn 2005, Johnson et al. 2006), 7-helix transmembrane receptors coupled to G-protein (Lau et al. 2004, Lortat-Jacob et al. 2002, Parish 2006) and other multiple-helices transmembrane receptors (Hacker et al. 2005, Sasaki et al. 2008).

Different protein cores of the heparan sulfate proteoglycans have been described regarding growth factor and chemokine signaling transduction. Among them, the syndecan family is the most extensively studied (Alexopoulou et al. 2007, Bartlett et al. 2007, Beauvais and Rapraeger 2004, Fears and Woods 2006, Lopes et al. 2006a, Porcionatto et al. 1999, Su et al. 2007, Tkachenko et al. 2005). Nevertheless, other cell surface proteoglycans, such as glypicans (Cano-Gauci et al. 1999, Capurro et al. 2008, Filmus et al. 2008, Gumienny et al. 2007, Kayed et al. 2006, Song et al. 2005, Traister et al. 2008) and betaglycans (Harrison et al. 2005, Lewis et al. 2000), have been also implicated with cellular transduction mechanisms.

Another possibility is that the proteoglycan itself could act as a transducer for cell signaling elicited by a growth factor. Using as a working model, with L6 myoblast cells lacking endogenous functional high-affinity FGF receptors, it was proposed the direct involvement of syndecan with the internalization of FGF-2 and cellular response. This cell signaling is distinct from the better known transmembrane tyrosine kinase receptors (Quarto and Amalric 1994).

Mechanisms leading to gene regulation can also involve interaction of the extracellular cell matrix (ECM) components with cytoskeleton via transmembrane surface receptors, such as integrins and/or heparan sulfate proteoglycans (Fig. 3B). Integrins consist of heterodimers of single helix transmembrane proteins that, like syndecans, do not display enzymatic activity and so their actions as transducers depend on the activation of a number of cytoplasmic kinases. The best evidence for a specific role of integrins in cell adhesion and cell migration comes from studies of focal adhesion formation. Fibronectin, vitronectin, collagen, laminin, among others, including matrix proteoglycans, such as perlecan, collagen XVIII and agrin, are potential ECM ligands of integrins. On the other hand, the intracellular domain interacts with many cytoplasmic proteins including talin, vinculin, paxillin and α-actinin. These set of molecules can activate kinases like FAK (focal adhesion kinase) and Src, which in turn leads to a cascade of protein phosphorylation that regulates genes expression involved in cell spreading, recognition, adhesion, growth control, apoptosis, etc. (Bernfield et al. 1999, Lopes et al. 2006a). The integrins thus link across the plasma membrane two networks: the extracellular and the intracellular actin filamentous system.

The connection of ECM and cytoskeleton can also be mediated by syndecans either directly or as co-receptor of integrins. The cytoplasmic region of syndecans contains two domains that are conserved in each of the four syndecans, and flank a central variable region that is distinct for each family member. The in-

variant region close to the transmembrane domain contains serine and tyrosine which are potential substrates for phosphorylation, as well as specific motifs that can bind to kinases as Src and Fyn. The variable region is distinct for each of the 4 family members. The function of this variable domain is largely unknown except for syndecan- 4, where it has been shown to bind PIP2 (4,5-bisphosphate phosphatidylinositol) and activate PKC-a, leading to oligomerization of the proteoglycan in focal adhesions. Several studies have shown that the oligomeric status of the cytoplasmic domain is related to the activation of the downstream signaling pathway. The other conserved domain, at the C-terminal of the protein, interacts with specific proteins containing PDZ domains, such as CASK and syntenin which are thought to link membrane components to the underlying actin-containing cytoskeleton. Interestingly, the variable and first conserved domains of syndecan-4 can also bind other proteins related to the cytoskeleton, such as syndesmos and α-actinin (Oh and Couchman 2004, Woods and Couchman 2001).

A direct role of syndecan-4 in focal adhesion regulation was observed using fibroblasts derived from syndecan-4 or fibronectin null mice. It was shown that the proteoglycan was capable to regulate FAK phosphorylation in a Rho dependent mechanism with no activation of PKC (Wilcox-Adelman et al. 2002). In a recent paper, a novel RGD-independent cell adhesion mechanism is proposed, in which syndecan-4 activates PKC-α and its subsequent interaction with the β1-integrin chain and, thus, initiating the FAK signaling cascade and actin-stress fiber organization (Telci et al. 2008). Recent results identify syndecan-4 as a novel receptor for the N-terminus of TSP-1 (thrombospondin) interfering with cell adhesion through activation of FAK (Nunes et al. 2008).

Figure 4 illustrates an experiment using confocal immunofluorescence microscopy, showing the co-localization of syndecan-4 and VEGF-receptor as well as that of syndecan-4 and FAK.

Synergistic control of cell adhesion involving integrins and syndecans were recently reviewed (Alexopoulou et al. 2007, Morgan et al. 2007).

It should also be referred that $\alpha 5\beta 1$ integrin is a part time proteoglycan, and the GAG chains play an essential role in the control of motility of cells on fibronectin and, thus, in the cascade of signaling events (Franco et al. 2001, Veiga et al. 1997).

Furthermore, depending on the biological process investigated as cell migration, adhesion, growth, differentiation and apoptosis, it has been found that the extracellular matrix heparan sulfate proteoglycans, such as perlecan (Baker et al. 2008, Farach-Carson et al. 2008, Farach-Carson and Carson 2007, Giros et al. 2007, Jiang and Couchman 2003, Knox and Whitelock 2006, Lindner et al. 2007, Smirnov et al. 2005), agrin (Fox and Zinn 2005, Glass et al. 1996, Jury et al. 2007, Ngo et al. 2007, Tourovskaia et al. 2008, Williams et al. 2008), collagen XVIII (Fjeldstad and Kolset 2005) and testican (Schnepp et al. 2005) can also modulate the activity of growth factors, cytokines, morphogens and enzymes.

The different cell ligands and receptors trigger downstream pathways that share cytosolic components, leading ultimately to the activation of a complex biomolecular network. This large network of molecular interactions and signaling pathways involve phosphorylation of key substrates including enzymes, microtubules, histones, and transcription factors that play pivotal roles in determining the cellular response.

The signaling systems evoked by the interaction of heparan sulfate proteoglycans with extracellular ligands and/or receptors include pathways such as Ras/Raf/MAPK (Leicht et al. 2007), PIP3/Akt (Carnero et al. 2008), PLC/PKC (Escriba et al. 2007), cAMP/PKA (Murray 2008, Wojtal et al. 2008), among others. They are of great interest and play a key role in normal cell behavior and in diseases such as cancer, arthritis and rheumatism.

PMA (phorbol 12-myristate 13-acetate) is recognized as a strong and specific activator of PKC mimicking diacylglycerol. PMA specifically stimulates the synthesis of syndecan-4 in endothelial cells in a mechanism mediated by PKC activation. The most remarkable aspect of these results, however, was the correlation between the up-regulation of heparan sulfate proteoglycans expression and the blockade of G1-S phase transition triggered by PMA (Moreira et al. 2004, Porcionatto et al. 1998, 1994).

Recently, it has been shown that over-expression of the EJ-*ras* oncogene in endothelial cells modifies the

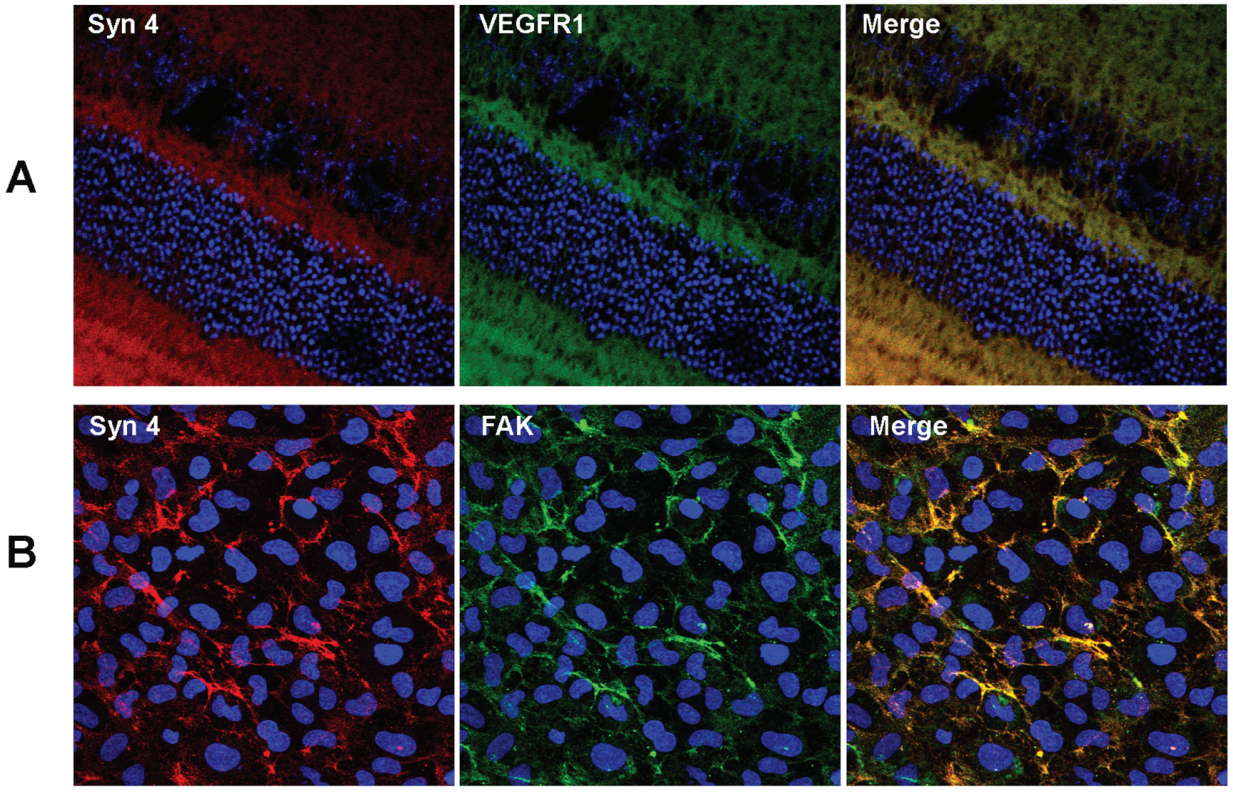

Fig. 4 – Confocal immunofluorescence microscopy showing syndecan-4 cellular localization and protein interactions. (A) Rat retina tissue was triple stained using a monoclonal anti-syndecan-4 (red), anti-VEGF receptor 1 (green) and DAPI for nucleus (blue). Merge represents the co-localization of syndecan-4 and VEGFR1. (B) Cultured rabbit aorta endothelial cells were triple stained using anti-syndecan-4 (red), anti-Focal Adhesion Kinase (green) and DAPI for nucleus (blue). Confocal imaging shows co-localization of syndecan-4 and FAK. Both figures depict syndecan-4 as a co-receptor.

cell cycle, up-regulates the expression of syndecan-4, and down-regulates several enzymes involved in heparan sulfate biosynthesis, leading to a decrease in the N- and O-sulfation of the chains (Lopes et al. 2006b). These results are in accordance to the structural characteristics of heparan sulfate from neoplastic tissues (Jeronimo et al. 1994, Oba-Shinjo et al. 2006).

The understanding of how cells control proliferation and differentiation, survival and death, migration and adhesion, requires the analyses of the crosstalk of the various pathways involved in these processes.

Although growth factor receptors are generally thought to carry out their role in signal transduction at the cell surface, many of these transmembrane proteins translocate to the nucleus after ligand stimulation.

In the 80's it was reported a nuclear pool of free heparan sulfate chains using radioactive sulfate labeling of a hepatoma cell line (Fedarko and Conrad 1986, Ishihara et al. 1986). Independently, the presence of FGF-2 in the nucleus was also documented using endothelial (Bouche et al. 1987) as well as CHO cells (Caizergues-Ferrer et al. 1984) in G_0-G_1 transition. On the other hand, the connection in the internalization of both heparan sulfate and FGF-2 was proposed using L6 myoblasts (Quarto and Amalric 1994).

Interestingly, the up-regulation in the expression of heparan sulfate proteoglycan induced by growth factors and PMA in endothelial cells occurs during the G_0-G_1 transition and has also been described associated with PKC pathway (Porcionatto et al. 1998, 1994). These results have been confirmed using corneal stromal fibroblasts (Hsia et al. 2003).

Lipid rafts seem to play an important role for FGF-2 and heparan sulfate proteoglycan internalization. FGF-2

induces syndecan-4 clustering of the proteoglycan, leading to the internalization by macropinocytosis of both molecules. It requires lipid rafts integrity, occurs in a nonclathrin-, non-dynamin-dependent manner and involves Rac1, which is activated by syndecan-4 clustering (Tkachenko et al. 2004).

The importance of glypican endocytosis as a positive or negative modulator is pointed out in the regulation of Hedgehog (Hh) signaling and in Wingless gradient formation (Beckett et al. 2008, Gagliardi et al. 2008).

Heparan sulfate proteoglycans have also been described in the internalization of ligands other than growth factors (Poon and Gariepy 2007). Syndecans and perlecan have been shown to mediate the clathrin-independent endocytosis of lipoproteins (Fuki et al. 2000, 1997). Also, a physiological role for glypican-1 in the cellular homoeostasis of polyamines was demonstrated in vesicle caveolae-mediated endocytosis (Belting 2003, Cheng et al. 2002).

Endocytic pathway for many cationic ligands mediated by cell surface proteoglycans involving raft-dependent macropinocytosis have been studied and proposed as a delivery of therapeutic genes and drugs to intracellular compartments (Fan et al. 2007, Nascimento et al. 2007).

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RESUMO

Proteoglicanos de heparam sulfato são encontrados tanto superfície celular quanto na matriz extracelular em todas as espécies animais. Esta revisão tem enfoque nas características estruturais dos proteoglicanos de heparam sulfato e nas interações destes proteoglicanos com proteínas que levam à sinalização celular. As cadeias de heparam sulfato, devido a sua variedade estrutural, são capazes de se ligar e interagir com ampla gama de proteínas, como fatores de crescimento, quimiocinas, morfógenos, componentes da matriz extracelular, enzimas, entre outros. Existe uma especificidade estrutural que direciona as interações dos heparam sulfatos e proteínas alvo. Esta especificidade está relacionada com a estrutura da cadeia do polissacarídeo e os motivos conservados da cadeia polipeptídica das proteínas envolvidas nesta interação. Os heparam sulfatos possuem papel na sinalização celular como receptores ou co-receptores para diferentes ligantes. Esta ligação dispara vias de sinalização celular levam à fosforilação de diversas proteínas citosólicas ou com ou sem interações diretas com o citoesqueleto, culminando na regulação gênica. O papel dos proteoglicanos de heparam sulfato na sinalização celular e vias de captação endocítica também são discutidas nesta revisão.

Palavras-chave: glicosaminoglicanos e interações com proteínas, fatores de crescimento, adesão focal, matriz extracelular, ciclo celular, proliferação celular.

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