Multiple alterations of heparan sulphate in cancer

O García-Suárez¹, I Fernández-Vega², LM Quirós³,⁴*®

Abstract
Introduction
Heparan sulphate is a complex polysaccharide that contains specific structural patterns generated through a series of interdependent enzymatic reactions that can involve various isoenzymes with differences in their specificities, responsible for the modification of the structure of heparan sulphate in physiological and pathological processes. The aim of this review is to discuss the multiple alterations of heparan sulphate in cancer.

Discussion
Tumour cells exhibit altered growth patterns and metabolism. This is supported by deregulated expression of multiple gene sets associated with tumour development and progression. Malignant cancers are characterized by their invasiveness and can even metastasize to distant locations in the body.

Heparan sulphate proteoglycans are implicated in these processes and can influence the growth, invasion and metastatic properties of cancerous cells. Cells exercise exquisite control over heparan sulphate composition and sequence, varying among cell types, development stages as a result of cell transformation in pathological processes. Numerous tumours show quantitative and structural alterations of heparan sulphate chains, largely caused by changes in the expression of the enzymes responsible for their biosynthesis, particularly at the fine structure level.

Conclusion
This article focuses on reviewing the type of changes that occur at different stages of heparan sulphate chain biosynthesis in various tumours. Altered transcription can occur in any phase of heparan sulphate biosynthesis, but is particularly frequent at the level of the family of the 3-O-sulphotransferases, and the families of the 6-O-sulphotransferases and extracellular sulphatases. The result is the alteration of the ligand binding sites responsible for directing specific cell signalling effects, facilitating tumour progression.

Introduction
Proteoglycans (PGs) are a diverse group of glycoconjugates composed of different core proteins posttranslationally modified with one or more covalently attached glycosaminoglycan (GAG) chains. GAGs are linear anionic polysaccharides consisting of repeating disaccharides. Heparan sulphate proteoglycans (HSPGs) comprise a reduced and specific group of proteins covalently linked to HS GAG chains (Figure 1A).

HS synthesis occurs mainly in the Golgi apparatus, and is initiated by the formation of a tetrasaccharide linkage on the protein core, synthesized by stepwise addition of xylose, two galactose units and a glucuronic acid residue. Subsequently, the HS chain is elongated by the addition of alternating D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine (GlcNAc). At various positions, the molecule is modified by a series of interdependent enzymatic reactions that include N-deacetylation of GlcNAc, usually followed by N-sulphation to produce GlcNSO₃, creating sulphated S-domains. Within these regions, GlcA can be epimerized to idurionate (IdoA), and O-sulphate groups can be added at C6 of GlcN and C2 of IdoA residues. Minor sulphations at C3 of GlcN and C2 of GlcA may also occur (Figure 1B). Chain modification results in clusters of highly sulphated regions (NS domains), alternating with non-sulphated (NA domains) and mixed (NA/NS) domains³–⁵.

HS species from different sources differ at the level of molecular size, distribution of the domains and modifications within these domains. Nevertheless, the overall patterns of chain modifications are cell specific, and appear to be largely independent of the core protein type²–⁵. Furthermore, the expression of the sequences of sulphated sugars can be spatially and temporally regulated in tissues, which is particularly evident in development⁶.

HS chains are able to interact selectively with different types of soluble and insoluble proteins, lipids and even microorganisms⁷. Binding takes place preferentially through electrostatic interactions, and is specific to sets of variably modified disaccharides, usually within the sulphated domains. Two types of sites have been described: interactions involving more specific HS structures that may depend on relatively rare modifications and interactions involving several sequences, provided that these were of sufficient overall charge density²–³.

* Corresponding author
Email: quiroslus@uniovi.es

¹ Department of Morphology and Cell Biology, University of Oviedo, Oviedo, Spain.
² Department of Pathology, Hospital Universitario Central de Asturias, Oviedo, Spain.
³ University Institute of Oncology of Asturias (IUOPA), Oviedo, Spain.
⁴ Department of Functional Biology, University of Oviedo, Oviedo, Spain.
⁵ Conflict of interests: none declared.

All authors abide by the Association for Medical Ethics (AME) ethical rules of decision.
Review

HSPGs are ubiquitously present in tissues, occurring in all cells that have been investigated. They are mainly associated with the cell surface and the extracellular matrix (ECM) and a variety of both normal and pathological functions have been ascribed to them, including cell adhesion and migration, organization of the ECM, regulation of proliferation, differentiation and morphogenesis, cytoskeleton organization, tissue repair, inflammation, vascularization and cancer metastasis.

HSPGs may play diverse roles in cancer and act as either inhibitors or promoters of tumour progression depending on the type and stage of cancer. The expression of HSPGs is markedly altered during malignant transformation and tumour progression, affecting both the PG core proteins and the GAG chains. HS biosynthesis is not a random process, depending mainly on transcriptional and translational control of expression of the enzymes involved, as well as possibly being influenced by other factors such as post-translational modification of proteins, modulation of catalytic activities and availability of precursor molecules. Substantial evidence of HS alteration has previously been described in various tumours, and the resulting tumour-specific HS fine structures are able to assist cancer cells to proliferate, invade neighbouring tissues and metastasize to distal sites away from the primary site. This article focuses on the multiple alterations in HS structures and biosynthetic machinery described in different types of cancer.

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

Synthesis of the saccharidic chain

As mentioned above, the biosynthesis of HS begins with the formation of a tetrasaccharide linkage region that comprises of β-xylene, β1-4 galactose,
β1-3 galactose and β1-3 glucuronic acid (Figure 2A). The first step of this process involves the transfer of a xylose residue from UDP-xylose to the hydroxyl group of a serine on the core protein. The characteristic attachment sites consist of a Ser-Gly dipeptide flanked by acidic residues, and the reaction is catalysed by two xylosyltransferases (XylT1 and XylT2) that show different efficiency and different expression patterns. The attachment of xylose is followed by the addition of two galactose residues, in separate reactions, catalysed by galactosyltransferases I and II (GalT1 and GalT2), and a glucuronic acid residue catalysed by GlcA-transferase I (GlcAT1). The xylose unit can be modified by phosphorylation and the galactose units by sulphation reactions, which appear to be involved in regulation of GAG synthesis. At this point, the process may follow two divergent paths, depending on whether the addition of a GalNAc residue takes place, in which case it shifts towards the synthesis of Chondroitin sulphate (CS), or GlcNAc to conduct towards the synthesis of HS (Figure 2A).

Addition of the first GlcNAc residue to the linkage tetrasaccharide is carried out by enzymes with GlcNAcT-I activity. Two members of the exostosin gene family have been described, EXT2 and EXT3, which possess GlcNAcT-I activity, although it has been described that the EXT2 transfer of a GlcNAc residue to a linkage region that is phosphorylated by a xylose kinase 1 terminates chain elongation. This suggests that EXT2 controls GAG biosynthesis, and the lack of it causes GAG overproduction associated with pathological processes. HS polymerization involves the consecutive addition of alternating GlcA and GlcNAc residues, mediated by the action of two main enzymes, GlcA-TII and GlcNAc-TII. The proteins EXT1 and EXT2 can perform both reactions although EXT2 polymerizing activity is weak (Figure 2B). They form an EXT1/EXT2 heterooligomeric complex in the

**Figure 2:** Heparan sulphate biosynthesis. A, initiation of the synthesis of heparan sulphate chains. Biosynthesis begins with the addition of a xylose to specific acceptor serine residues of a PG core protein; the reaction is catalysed by two xylosyltransferases, XylT1 and XylT2. Subsequently, the biosynthesis continues with the stepwise addition of two galactose units, catalysed by GalT1 and GalT2, and a glucuronic acid residue by GlcAT1. HS chain extension requires the subsequent transference of a GlcNAc residue, while the addition of a GalNAc directs the pathway towards the biosynthesis of CS. During synthesis of the linkage region, a transient phosphorylation of the Xyl residue occurs, catalysed by a xylose kinase, which is dephosphorylated before subsequent polymerization. If persistent xylose phosphorylation occurs, the transference of a GlcNAc residue, catalysed by EXT2, terminates chain elongation. B. After synthesis of the linkage region and the addition of a GlcNAc residue, heparan sulphate biosynthesis involves copolymerization of GlcA (●) and GlcNAc (□) residues. Simultaneously, a series of modification reactions take place including epimerization of GlcAtoldoA (◇) and sulphation.

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

Golgi apparatus yielding a more active, biologically important enzyme form\(^1\).

The glycosyltransferases (GTs) involved in the biosynthesis of HS have been related with tumours. Thus, the genes EXT1 and EXT2 are tumour suppressors, associated with hereditary multiple exostoses (HME), characterized by the development of benign skeletal tumours in patients\(^1\). It has also been suggested that the three EXT-like genes, EXTL1-EXTL3, may each be a tumour suppressor, although none is linked with HME\(^1\). There is less information concerning the synthesis of the linker although there are certain studies that have suggested a contribution of overexpression of GalT1 to cancer development and metastasis\(^1\)\(^1\)\(^9\). However, numerous studies show quantitative alterations in the absolute levels of HS and CS in different tumour processes\(^1\)\(^2\)\(^3\)\(^4\), which may be associated with alterations in expression levels of the GTs as in associated regulatory enzymes.

**N-deacetylation/N-sulphation**

As the polymer forms, the first reaction involved in polymer modification is catalysed by a dual-activity N-deacetylase/N-sulphotransferase enzyme (NDST). NDST displays two effects: N-deacetylase activity removes the acetyl group from the GlcNAc residue to yield GlcNH\(_2\) while N-sulphotransferase activity transfers a sulpho group to the primary amine to form a GlcNS residue. These reactions create regions of contiguous N-sulphated disaccharides (NS domains) and regions of alternating N-sulphated and N-acetylated disaccharides (NA/NS domains) although a significant portion (40-70\%) of the HS chain remains unchanged (NA domains)\(^1\)\(^2\)\(^3\)\(^4\) (Figure 3). N-sulphation plays a key role in HS biosynthesis and it has been described as a prerequisite for subsequent modifications, which occur mainly in N-sulphated regions although 6-O-sulphated groups can also be found in cells lacking N-sulphated HS\(^1\)\(^4\).

Four human NDST isoforms have been identified and cloned: NDST-1–4 (Figure 1B). NDST-1 and NDST-2 mRNAs show broad overlapping tissue distribution while NDST-3 and NDST-4 are more restricted and are expressed primarily during embryonic development. NDST-3 and NDST-4 have been found to possess different enzymatic properties to NDST-1 and NDST-2; NDST-3 has much greater deacetylation activity compared to its sulphotransferase activity, whereas NDST-4 displays weak deacetylase activity but high sulphotransferase\(^1\)\(^5\).

Despite the relevance of NDSTs in the generation of the sulphated domains in the HS structure, references in the literature to altered expression of NDSTs in tumours are scarce. However, some examples have been referred to such as the up-regulation of NDST-1 and NDST-2 in hepatocellular cancer\(^1\)\(^6\) or the detection of NDST-4 transcripts in 50\% of invasive breast ductal carcinomas\(^1\)\(^7\). An added difficulty in analysing the expression of these enzymes in tumours results from the fact that it has been described that the expression of the NDST isoforms may be both translationally and post-translationally regulated\(^2\).

**Epimerization**

Further modifications of HS chains include the action of C5-GlcA epimerase. This enzyme uses the partially N-sulphated polysaccharide as substrate, and transforms some of the GlcA residues into IdOA by epimerization of the C5 carboxyl group (Figure 2B). The generation of IdOA residues is restricted to N-sulphated domains, since GlcA residues may be recognized as substrates if they are linked to an N-sulphated unit at the non-reducing end. This modification of the uronic acid residue profoundly affects the conformational and dynamic properties of the resulting polymer. The flexibility of the IdOA is important for specific interactions with protein ligands and depends

---

**Figure 3:** Heparan sulphate domain architecture. The highly sulphated S-domains are flanked on each side by less sulphated transition zones. In turn, these sulphated regions are separated by non-sulphated NA-domains.

---

*For citation purposes: Garcia-Suárez O, Fernández-Vega I, Quirós LM. Multiple alterations of Heparan sulphate in cancer. OA Cancer 2013 May 01;1(1):1.*
both upon its own substitution with 2-O-sulphate and on the substitution of adjacent glucosamine residues\textsuperscript{18}.

It has been suggested recently that epimerase is a relatively late evolutionary development and that its specificity, acting at an early point in the HS biosynthesis to convert GlcA-GlcNS to Idoa-GlcNS in preference to converting GlcA-GlcNAc to Idoa-GlcNAc, could constitute the basis for the relative abundance of these disaccharides\textsuperscript{19}. However, there are few references to GlcA epimerase alterations in cancer although some data have been cited, such as significant down-regulation of its expression in human breast tumours, suggesting the genes possible involvement in carcinogenesis\textsuperscript{20}.

O-sulphation

During the biosynthetic process of the HS chains, different combinations of O-sulphate groups can be added to the C2 of uronic acid and to C6 and C3 of the glucosamine residues (Figure 1B).

2-O-sulphation is closely associated with epimerization. The reaction is catalysed by a 2-O-sulphotransferase (2-OST) that, like C5-epimerase, occurs in one single isoform. The enzyme displays a greater affinity for Idoa-containing disaccharides than for GlcA-containing disaccharides, such that most Idoa units are sulphated at the C2, whereas 2-O-sulphated GlcA is rare\textsuperscript{1-3}. Physical and functional association has been described between GlcA C5-epimerase and Idoa 2-O-sulphotransferase, and 2-O-sulphation locks Idoa residues in the interaction-prone configuration\textsuperscript{2}.

There is some evidence that this enzyme can be associated with tumour progression. This is the case in prostate cancer where it is overexpressed in tumours and cell lines; 2-OST is essential for proliferation and invasion, and its expression correlates with increasing metastatic potential. It has also been suggested that up-regulation of 2-OST expression could produce a more efficient form of highly sulphated perlecan in metastatic prostate cancer tumours that fail to show increase in perlecan protein levels\textsuperscript{21}

Additional changes in the fine structure of the polysaccharide involve O-sulphation at C6 and C3 of glucosamine residues. As opposed to the 2-O-sulphation, performed by a single enzyme, 10 different isoforms are responsible for these two reactions, giving them an essential role in the generation of plurality of protein binding sites.

There are three forms of 6-O-sulphotransferases, 6-OST-1-3 (Figure 1B), which show differences in their substrate specificities and tissue expression\textsuperscript{1-3}. Alterations in the expression levels of one or more of these isoforms have been referenced in different tumours. Furthermore, strong expression of 6-OST-1 and overexpression of 6-OST-2 have been described in ovarian cancer although the HS molecules produced subsequently act as substrate for extracellular enzymes which modulate their biological function\textsuperscript{22}. Up-regulation of 6-OST-2 has also been mentioned in relation to human colorectal cancers in conjunction with alterations in the expression levels of some syndecans, while the predominant form present in normal mucous is 6-OST-3\textsuperscript{3}. Moreover, 6-OST-1 is up-regulated in hepatocellular cancer although the overexpression of extracellular sulphatases resolves in modest 6-O-undersulphation of HS\textsuperscript{16}. However, in other tumour types sub-expression of these enzymes has been described; thus, 6-OST-1-3 are down-regulated in glioblastoma, although the alterations in the expression of some PGs and HS biosynthetic enzymes in these tumours is altered in a subtype-specific manner\textsuperscript{23}.

The last family of enzymes that are involved in the modification of HS are the 3-O-sulphotransferases. The relevance of these enzymes is in the fact that they form the largest group, comprising seven different members (Figure 1B), and they are implicated in the formation of specific HS motifs that interact in a selective manner with specific protein ligands. The different proteins of this family have distinct temporal and spatial expression patterns, such that they seem to modulate several distinct developmental processes\textsuperscript{1-3}. Numerous studies have described alterations of these proteins in different tumours. Some up-regulations have been reported, as is the case of 3-OST-1 or 3-OST-3A in hepatocellular cancer and glioblastoma, respectively\textsuperscript{16,23}. However, in many cases sub-expressions of different isoforms have been determined; methylation-associated silencing of 3-OST-2 has been described in breast, colon, lung and pancreatic cancers\textsuperscript{24}, and in human malignant melanoma\textsuperscript{25}. Hypermethylation of 3-OST-2, as well as 3-OST-1 and 3-OST-3A, appears in chondrosarcoma cells, contributing to the invasive phenotype\textsuperscript{26}. Furthermore, down-regulation of isoforms 3-OST-4, 3-OST-5 and 3-OST-6 have been described in invasive breast ductal carcinomas\textsuperscript{17}.

Extracellular endosulphatases

Certain specific modifications of the patterns of sulphation of HS chains may take place after their biosynthesis and extracellular translocation. 6S modification is the only sulphate moiety known to be post-synthetically edited from the HS chains. This reaction is performed by two endosulphatases, Sulf-1 and Sulf-2 (Figure 1B), located at the cell surface, which remove glucosamine-6S groups from specific regions of the chains. Since 6S modifications are directly linked with the regulation of certain signalling events, the function of these enzymes is to fine-tune the sulphation patterns of HS and thus modulate cell signalling through pathways requiring these coreceptors\textsuperscript{27}. There are many reported data that describe alterations in the expression of these enzymes in cancer:
Sulf-2 is up-regulated in various tumours such as glioblastoma, breast, hepatocellular or pancreatic carcinoma\(^1\). Sulf-1 has been described as up-regulated in some tumours such as glioblastoma, ovarian, breast, hepatocellular or pancreatic carcinoma\(^1,16\).\(^17\), while other reports indicate a down-regulation in ovarian, head and neck, breast or hepatocellular carcinomas\(^17\). The existence of alternative splicing variants of these enzymes has been described and related to divergences in expression in some tumours\(^19\).

**Conclusion**

HS chains display highly variable structures in their sulphated sequences that can be dynamically altered by cells in response to physiological or pathological conditions. These structures are synthesized sequentially by the action of different families of enzymes. In many tumours, significant alterations in the structure of HS emerge, largely caused by abnormalities in the regulation of the transcription of some of these biosynthetic enzymes. Altered transcription can occur in any phase of HS biosynthesis, but is particularly frequent at the level of O-sulphations of C3 of glucosamine, controlled by the family of the 3-O-sulphotransferases, and O-sulphations of C6 of glucosamine, controlled by the joint action of the families of the 6-O-sulphotransferases and extracellular sulphatases. The result is the alteration of the ligand binding sites responsible for directing specific cell signalling effects.

**Abbreviations**

CS, Chondroitin sulphate; ECM, Extracellular matrix; GAG, Glycosaminoglycan; GalNAc, N-acetyl-D-galactosamine; GlcA, D-glucuronic acid; GlcN, D-glucosamine; GlcNAc, N-acetyl-D-glucosamine; GT, Glycosyltransferase; HME, hereditary multiple exostosis; HS, Heparan sulphate; HSPG, Heparan sulphate proteoglycan; IdoA, Iduronic acid; PG, Proteoglycan

**Acknowledgements**

The University Institute of Oncology of Asturias is supported by Obra Social Cajastur, Asturias, Spain.

**References**

Review


Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)