Biochimica et Biophysica Acta 1840 (2014) 2491–2497

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Nuclear translocation of heparan sulfate proteoglycans and their functional significance☆

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ARTICLE INFO

Article history:
Received 4 December 2013
Received in revised form 17 April 2014
Accepted 18 April 2014
Available online 26 April 2014

Keywords:
Heparan sulfate
Proteoglycan
Nuclear translocation
Proliferation
Gene transcription

ABSTRACT

Background: Heparan sulfate proteoglycans (HSPGs) are important constituents of the cell membrane and they act as co-receptors for cellular signaling. Syndecan-1, glypican and perlecan also translocate to the nucleus in a regulated manner. Similar nuclear transport of growth factors and heparanase indicate a possible co-regulation and functional significance.
Scope of review: In this review we dissect the structural requirement for the nuclear translocation of HSPGs and their functional implications.s
Major conclusions: The functions of the nuclear HSPGs are still incompletely understood. Evidence point to possible functions in hampering cell proliferation, inhibition of DNA topoisomerase I activity and inhibition of gene transcription.
General significance: HSPGs influence the behavior of malignant tumors in many different ways. Modulating their functions may offer powerful tools to control fundamental biological processes and provide the basis for subsequent targeted therapies in cancer. This article is part of a Special Issue entitled Matrix-mediated cell behaviour and properties.

1. Short history of proteoglycan discovery

Polymeric acid carbohydrate moieties were recognized in the late 19th century as major components of connective tissue matrices. It was then demonstrated that these structures are linked to proteins, a finding that was not elaborated on for many years. Biochemical analysis of these so-called "mucopolysaccharides", later called "glycosaminoglycans" (GAGs) identified their structure as polymeric disaccharides. These repeating units consist of an amino sugar and an uronic acid or neutral sugar. The structure may also include one or more O-linked, and less frequently N-linked sulfate groups, which together with the uronic acid give the polysaccharide a strongly polyanionic character. Based on the structural features, the different GAGs were categorized as heparan sulfate (HS), dermatan sulfate (DS), chondroitin sulfate (CS), keratan sulfate and hyaluronic acid (HA). HA, now called hyaluronan, lacks sulfate groups and it differs from the others in molecular size and biosynthetic pathway.

The most abundant and therefore first studied GAG is CS that is present in connective tissues, whereas other GAGs including HS could be isolated from a large number of cellular tissues such as the liver. In fact, these molecules have been isolated from the cell surface glycocalyx [1]. A modification of HS, heparin was shown to interact with blood coagulation, and has gained widespread clinical use [2]. The biological functions of GAGs were generally less characterized. Because of their large molecular size and strongly polyanionic nature, they were considered spacers, regulating the transport of ions through tissues and mineral precipitation.

The proteoglycan (PG) nature of these mucopolysaccharides was thus not established until the sixties. It was only in the eighties when the revolution of molecular biology provided tools to reveal the structure of the various protein cores. Since then the PGs are classified according to their protein core. The combination of molecular biology and immunology made it possible to gain understanding of their localization and much of their function. The role of PGs in living organisms is, however, far from completely clarified. One reason for this is the enormous structural heterogeneity in their carbohydrate chains. The diversity rests in the length of the GAG chain, its sulfation at different positions, and the presence of various uronic acid epimers. This variety in negative charges results in a tremendous variability in polarity and tertiary structures of the chains, thereby influencing their ability to interact with surrounding molecules.

Later PGs have been identified as active participants of physiological and pathological events. They form concentration gradients during embryonic development, which, in turn, determine the structural assembly of tissues and organs. Depending on their structure and localization, PGs

☆ This article is part of a Special Issue entitled Matrix-mediated cell behaviour and properties.

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http://dx.doi.org/10.1016/j.bbagen.2014.04.015
0304-4165/

are not only space filling structural tissue components, but also interfere with signal transduction, influence hemostasis, inflammation and regeneration. Thus, they are important factors during tumor formation and progression [3,4]. Notably, various stimuli, such as inflammation or cell injury, initiate characteristic shedding of transmembrane proteoglycans. The released fragments retain the regulatory functions of the polysaccharide chains and they exert PG actions at distance from the cell surface, in the surrounding extracellular matrix or in the systemic circulation [5].

It is still unclear what determines which GAG chain will be synthesized on the core protein. In general, the majority of CS-DS PGs reside in the extracellular matrix, whereas most cellular PGs carry HS chains. As HS and DS contain iduronic acid (conferring additional sulfation and altered tertiary structure to these molecules) PGs-glycanated with these two types of GAG are more prone to molecular interactions.

In the late eighties significant efforts were devoted to study the fine structure of HS chains and specific sequences that interact with particular protein structures. A number of interactions have been revealed, often involving a HS stretch of 5–6 monosaccharides. HS binds a large number of ligands including ECM components and cell-surface adhesion molecules [6,7], chemokines [8], growth factors and growth factor receptors [9–11]. The best studied ligation reactions are those involving the activation of antithrombin III and the binding of FGF-2 to its cell surface receptor [12]. It is well documented that HS interacts with both FGF2 and FGF receptor-1 (FGFR1) thereby forming a ternary complex that exerts efficient cell growth signaling properties [9].

2. Presence of heparan sulfate in the cell nucleus

The identification of heparan sulfate in the cell nucleus and its potential regulatory role in cell proliferation were reported as early as 40 years ago. This unusual localization of GAG chains alone, or as part of PG molecules, however, became generally accepted only quite recently. Accumulating evidence suggests possible functions of nuclear HS in cell differentiation and proliferation although they may carry additional regulatory properties. The first experiments indicating the presence of HS in the cell nucleus date back to 1974, when Kinoshita showed that the mucopolysaccharide in the cell nucleus localizes in the template active part of the chromatin and augments of RNA synthesis [13]. The following year it was reported that mucopolysaccharides stimulate transcription by making new RNA polymerase binding sites available on the chromatin [14]. Scientists managed to isolate CS and HS from purified nuclei of brain tissue and melanoma cells [15,16]. Two papers were published in 1986 [17,18] describing the link between transport of HS to the nucleus and inhibition of cell division [19]. Following metabolic labeling with sulfate, Ishihara and Fedarko detected free HS in the nuclei of cultured hepatoma cells. This HS was more abundant in confluent cells representing slightly more than 6% of the total cell-associated HS. The structure of this nuclear HS was similar in proliferating and confluent cells, but differed from that of HS isolated from other cell compartments. Disaccharide analysis revealed that nuclear HS carries a sulfated glucuronic acid residue as a unique structural feature. Although the precise structure of this HS was unknown, it was hypothesized that this GAG might interact with histone proteins. Any functional effect of the nuclear HS was, however, not established at the time of the report.

In 1992 Busch *et al.* observed that heparin interferes with the TPA induced jun/fos AP1 mediated transcription, concluding that nuclear GAGs can exert similar effects [20]. Shortly thereafter Kovalszky *et al.* discovered that nuclear HS co-localizes with FGF-2 [21], and subsequently it was shown that an antiproliferative HS can be detected in fibroblasts [22]. This HS was rich in L-iduronic acid, and its presence in cell nuclei correlated with inhibition of cell proliferation.

3. General feature of proteoglycans detected in the nucleus

PGs are ubiquitously present in the extracellular matrix, or on the cell surface, either as transmembrane proteins, such as the syndecans, or covalently bound to the plasma membrane, like the glypicans. During the last decade it has become evident that PGs can also be present in the cell nucleus. While the scientific community slowly accepted the existence of HS in the nucleus, the presence of proteoglycans was still debated in 2001–2002. Finally two independent reports verified the presence of syndecan-1 and HSPG in the nucleus of various tumor cells and stromal fibroblasts [23,24]. These PGs, however, translocate from other cell compartments, and no PG has been constitutively found inside the cell nucleus.

3.1. Syndecans

Syndecans constitute a family of 4-transmembrane HS proteoglycans (HSPGs) that are typically present on the cell surface [10,25], although they have also been found in the cell nucleus [23] and in shed forms in blood [26–29]. Each syndecan is expressed in a highly regulated cell-, tissue- and development-specific manner [11,30]. Syndecan-1 is the major syndecan on epithelial cells [25], syndecan-2 is present on mesenchymal cells [31], syndecan-3 in neuronal tissue and cartilage [32,33] while syndecan-4 is expressed in most cell types [34,35].

Syndecan-1 has been detected in the nuclear compartment of a wide range of cancer types including malignant mesothelioma, multiple myeloma, breast carcinoma, lung adenocarcinoma and neuroblastoma [24, 36–38]. Syndecan-2 has been identified in the nuclei of injured cerebral cortex neurons and astrocytes [36] and in chondrosarcoma [37].

The core proteins of syndecans consist of a C-terminal cytoplasmic domain, a transmembrane domain and an N-terminal extracellular domain [11,39]. While the single-pass transmembrane domain is highly conserved, the ectodomains vary in length and in amino acid sequence and contain conserved motifs for GAG attachment, cell interaction, proteolytic cleavage and oligomerization. The cytoplasmic region binds cytoskeletal and PDZ-domain proteins, thus influencing the dynamics of the actin cytoskeleton and membrane trafficking. These interactions control syndecan recycling through endosomal compartments, promote internalization of accompanying protein cargo, and regulate cell adhesion and various signaling systems [40–42].

The intact ectodomain of syndecan-1 is constitutively shed from the cell surface by endogenous proteolytic cleavage [30,43] as part of normal cell surface PG turnover [44]. Elevated levels of soluble syndecan-1 ectodomain have been demonstrated in sera from patients with lung cancer [28], multiple myeloma [27] and Hodgkin's lymphoma [29]. Shedding can be accelerated by a variety of physiological stimuli, including growth factors, chemokines, bacteria, and cellular stress [45]. Recent studies showed that heparanase enhances syndecan-1 shedding by stimulating the expression of the active protease, thereby stimulating tumor growth and spread [46,47]. The release of the syndecan-1 ectodomain has functional consequences. While membrane-bound syndecan-1 may stimulate proliferation and inhibit invasiveness of tumor cells, overexpression of a constitutively shed syndecan-1 decreases the proliferation and promotes the invasiveness of cancer cells [48].

It is noteworthy that heparanase and syndecan-1 interact not only on the cell surface but they also co-localize in the nucleus. It seems that the sub-cellular localization of syndecan-1 might be crucial for its function and the nuclear translocation adds further complexity that needs to be further addressed in the context of variably differentiated tumor components.

3.2. Glypicans

The glypican family consists of six members of HS proteoglycans. Although they reside on the surface of epithelial cells, they are attached to the cell surface by their C termini, which are covalently linked to glycosyl-phosphatidyl-inositol (GPI) molecules. The glypican core proteins are similar in size, three-dimensional structure and they have a conserved domain of 14 cysteine residues in identical location. Unlike

syndecans, the HS chains of glypicans are located close to the cell membrane. Glypican has been detected in the cell nucleus of glioma cells as well as in neurons. C6 glioma cells are characterized by prominent nuclear immune reactivity in G1 phase, indicating their decisive role in cell cycle progression [38].

Family members of the glypicans play important roles in the morphogenesis and participate in the Wnt and Hedgehog signaling pathways. Similarly to syndecans, they act as co-receptors for various cell surface receptor molecules. Glypican expression, especially that of glypican 1 and glypican 3, can be affected in various tumors; thus, these molecules are designated as oncofetal proteins [49].

3.3. Perlecan

Perlecan is the characteristic HSPG of the basement membrane. It is a large multi-domain protein with three HS chains synthesized by endothelial and smooth muscle cells. Perlecan interacts with a wide variety of ECM proteins as well as cell surface molecules, and it is a key component of the endothelial barrier [50]. Endorepellin derived from the C terminal domain of perlecan is a potent angiogenesis inhibitor [51].

In contrast to other HSPGs little is known about the role of perlecan in the nucleus. Its nuclear presence could be revealed in astrocytes after injury in the mid-phase of regeneration but not in normal brain tissue [36]. Perlecan is associated to FGF-2 and thereby it stimulates angiogenesis. Notably, FGF-2 is one of the key growth factors implicated in brain regeneration stimulating the proliferation of astrocytes and neurons in an autocrine or a paracrine manner, respectively. To this end, FGF-2 sequentially utilizes different types of HSPGs, including syndecan-2, glypicans and perlecan [52].

3.4. CD44

The CD44 gene contains 20 exons. Ten of the exons are alternatively spliced to encode a portion of the ectodomain, generating numerous CD44 spliced variant isoforms ($CD44_V$) in some epithelial tissues and several cancers. The standard CD44 ($CD44_S$), lacking all exon variants, is widely expressed in most cell types. CD44HSPG contains the V3 variable exon that contains SGSG amino acid repeats; the essential structure for glycanation. Recently it was found that CD44 HSPG is implicated in the maintenance of cancer stem cell (CSC) phenotype, by interacting Oct4-Sox2-Nanog transcription factors leading to both formation of a complex and nuclear translocation of the three CSC transcription factors [53]. It seems that the proteoglycan itself can internalize, as upon engagement by its ligand, a portion of its intracellular domain translocates to the nucleus. This translocation proved to be transportin-dependent, whereas inhibition of beta-importin failed to interfere with the nuclear uptake of CD44 [54].

4. Structural requirements for nuclear translocation

Nuclear transport pathways of macromolecules engage unique and shared components, often acting together in an orchestrated manner [55,56]. The nuclear import requires the presence of a nuclear localization signal (NLS) corresponding to a short peptide, but there are also NLS-independent mechanisms [57]. The minimal sequence required for the nuclear translocation of syndecan-1 is the conserved juxtamembrane RMKKK motif, which serves as a nuclear localization signal (NLS), important for tubulin-dependent nuclear translocation of syndecan-1 [58]. Replacement of the arginine in the RMKKK sequence causes a dramatic decrease of the proportion of cells with nuclear syndecan-1, pointing toward the critical involvement of arginine in this nuclear translocation. Deletion of this highly conserved sequence (that is present also in syndecan-1, -3 and -4) prevents the nuclear translocation of syndecan-1 [59]. Interestingly, the MKKK sequence is additionally involved in raft-dependent endocytosis.

The nuclear localization signal of glypican KRRR (G/A) K is located upstream of the HS attachment site. This region appears to be blocked by HS chains. Removal of the HS chain exposes the NLS to the nuclear import system and may redirect the core protein to the cell nucleus [60]. In case of standard CD44 a bipartite nuclear localization signal (NLS) was mapped to the cytoplasmic tail of CD44, which mediates its nuclear translocation. As the same region is present on the CD44V3 HSPG splice variant, it is conceivable that this form utilizes the same ^{292}RRRCGQKKK^{300} NLS. CD44 is internalized through endosomal sorting and imported to the nucleus through the nuclear pore complex [61].

5. Regulation of nuclear translocation

Observations of nuclear translocation of PGs have triggered investigations to uncover the underlying mechanisms and to raise several important questions. Is HS present in the nucleus alone, or it is attached to a particular core protein? How are these molecules targeted to the nucleus? Do they first leave the cells where they have been synthesized or is the nucleus their primary target? How is the shuttling of PGs regulated?

Right now we are only at the beginning of understanding that PGs are able to enter the cell nucleus and act there as regulators of nuclear processes. The nuclear translocation of syndecan-1 occurs in a time- and tubulin-dependent manner in various benign and malignant cells. Syndecan-1 co-localizes with tubulin in the mitotic spindle in all phases of mitosis, and interference with tubulin integrity hampers the transport of syndecan-1 to the nucleus [23]. The reactivity to this PG is initially present in the cytoplasm. Subsequently it becomes detectable in the cell nucleus and nucleolus before it can be observed in the plasma membrane, this latter reactivity being mainly present at cell–cell contact sites. In the nucleus the ectodomain co-localizes with both endodomain and HS, indicating that the entire molecule is present.

The amount of HSPG core protein in the cell nucleus is increased when the cells grow on a fibronectin matrix instead of collagen. Inhibition of protein kinase C (PKC) results in an increase of nuclear HSPG, whereas PKC stimulation with phorbol 12-myristate 13-acetate (PMA) inhibits the nuclear targeting of HSPG [24]. This indicates that PKC can regulate the actual amount of nuclear HS. It has also been suggested that nuclear translocation of HSPG is dependent upon dephosphorylation of their protein core, triggered by FGF-2 [62].

A critical factor regulating the level of both HS and syndecan-1 in the nucleus is heparanase, an endoglycosidase that cleaves HS and causes a dramatic reduction of the amount of nuclear syndecan-1 levels in a concentration-dependent manner [63]. This observation suggests that the HS chains are important factors for the nuclear translocation or degradation of syndecan-1. Overexpression of heparanase in many cancer types is associated with poor survival [64–67]. In addition to its localization in the cytoplasm and cell membrane, heparanase is identified in the nuclei of normal and malignant epithelial cells [68–70] and the nuclear heparanase seems to be related to cell differentiation [71]. The decrease or loss of nuclear syndecan-1 in heparanase overexpressing myeloma cells is concomitant with a simultaneous increase in histone acetyltransferase (HAT) activity. This will in turn enhance the transcription of a number of proteins such as MMP-9, VEGF, HGF and RANKL, which all drive an aggressive tumor phenotype [72]. Conversely, restoration of the syndecan-1 level diminishes this HAT reactivity, pointing to an inhibitory role of nuclear syndecan-1.

6. Nuclear functions

The functions of the nuclear HS and PGs are, however, still incompletely understood. Many of the known nuclear functions are mainly attributed to HS interaction with growth factors and various nuclear structures. Although syndecan-1 seems to be present in the nucleus as an intact molecule, we cannot exactly determine if the active nuclear form is a PG or a free GAG. Evidence points to possible functions in the

shuttling of FGF-2 into the nucleus [62], inhibition of DNA topoisomerase I activity [21] and control of cell proliferation and differentiation [19].

6.1. Nuclear delivery of growth factors and macromolecules

Over the past decades convincing evidence accumulated for the nuclear translocation of growth factors and cytokines [73–75]. The majority of growth factors with nuclear translocation bind to HSPGs, which are efficient vehicles for intracellular delivery of genes and macromolecules [60,76–84]. A multitude of ligands, viruses, growth factors, morphogenes, cell penetrating peptides, nucleic acid complexes [78,80], lipoproteins and exosomes [85] enter the cells through HSPG-mediated endocytosis [86]. The juxta-membrane MKKK motif is the minimal sufficient structure for efficient raft dependent endocytosis [87]. The same sequence is part of the RMKKK nuclear localization signal of syndecan-1 [58]; thus, this motif seems to be crucial both for endocytosis and nuclear transport.

Notably, both syndecan and glypican mediate uptake of nanoparticles, and despite their different membrane anchorage, they govern these nanoparticles to the same vesicular compartment during the early steps of endocytosis [81]. These HSPGs act as internalization receptors for macromolecular cargos and are important mediators of intracellular trafficking and endosomal processing. The end-point of receptor internalization is endolysosomal degradation of the ligands and their corresponding receptor. Alternative mechanisms comprise escape from degradation and receptor recycling to the plasma membrane or nuclear translocation. Escape from lysosomal degradation has been proposed [73] and subsequently nuclear co-localization of growth factors and HSPG has been shown by independent investigators. In this context the HS might well be the critical factor protecting the growth factor from lysosomal degradation because a mutual protective role has been suggested between HS and FGF-2 [88,89].

FGF-2 was amongst the first, and most extensively studied, molecules shown to interact with HS on the cell surface [88,90] and in the nucleus [52,91]. The mechanism of FGF-2 shuttling is multifactorial, comprising both FGF receptor [92] and HSPG mediated nuclear translocation [58,62,93,94]. Syndecan-1 is clearly implicated in the nuclear delivery of FGF-2, the two compounds sharing the same tubulin-mediated transport route to the nucleus, where they co-localize with heparanase. In contrast, FGFR-1 seems to follow a different transport route and remains mainly in the peri-nuclear area [69].

6.2. Inhibition of topoisomerase-I activity

The physical characteristics of HS, namely their negative charges, make these molecules suitable for actions inside the cell nucleus. HS can compete with DNA for interactions with proteins such as transcription factors and DNA-binding enzymes. It has been demonstrated *in vitro*, that HS not only bind to, but also inhibit topoisomerase-I activity, which is important for the unwinding of supercoiled DNA during transcription. Gel shift assays show that HS not only competes for topoisomerase-I but it is able to dissociate the DNA– topoisomerase-I complex.

This inhibition of topoisomerase-I activity by HS is dose-dependent and its efficacy depends upon the source of GAG. HS isolated from normal liver appear to be much more efficient in topoisomerase inhibition than that isolated from hepatocellular cancer [21]. This finding together with the observation that FGF-2 abrogates the HS-topoisomerase interaction indicates that the outcome of interaction depends on the fine structure of the carbohydrate chain. Disaccharide analysis of these HS species, however, could not uncover major differences, suggesting an importance of the disaccharide sequences [95]. In support, it has been shown that the effect of HS on topoisomerase-1 is modulated by nuclear heparanase. Activation of EGFR initiates nuclear translocation of heparanase and degradation of HS, resulting in increased activity of topoisomerase-I. This regulatory cascade represents a new mechanism for EGFR-induced cell proliferation [96]. Experiments with biotinylated HS show that it is easily taken up by hepatoma cells [97]. The effects of free HS on topoisomerase-I activity corroborate that the process depends on the presence of the GAG chain regardless if bound to a protein or not. This inhibition of topoisomerase suggests that the presence of HS in the nucleus may inhibit gene transcription [21].

The picture, however, appears to be more complex. Not only topoisomerase-I but also topoisomerase-II can be influenced by heparin and with HS isolated from normal liver. Furthermore, when inside the nucleus, topoisomerase-I and FGF-2 compete for HS binding in a dose-dependent manner. As HS weakens the interaction between topoisomerase-I and DNA it can also interfere with camptotecin, a topoisomerase-I inhibitory drug whose mechanism of action requires physical contact between DNA and the enzyme. In this way heparin hampers the camptotecin-induced fragmentation of linearized plasmid DNA (Kovalszky, personal communication).

6.3. Regulation of nuclear proteins and transcription factors

The functional role of GAGs and PGs inside the nucleus is not restricted to topoisomerases. There are several candidate proteins supposedly regulated by HS. Thus, various transcription factors, casein kinase II, midkines and histones have all been reported as target molecules [97]. Inhibition of kinase activity may lead to decreased phosphorylation of nuclear proteins, including topoisomerase II or p53. Jun-Fos mediated transcription is repressed by heparin in a reporter gene assay. This was the first demonstration of the direct effect of heparin on the PKC/MAPK/Jun/Fos/AP1 signal transduction pathway. Later it was shown that heparin induces posttranslational modification of Jun-B [98].

HS interacts with several transcription factors and inhibits the binding of consensus oligo-DNA to AP1, Ets1, Sp1, TFIID and NFkB. HS preparations from benign and malignant hepatocytes have different inhibitory potentials, indicating that the binding specificity depends on the HS fine structure. Interestingly, liver carcinoma HS are less effective inhibitors of transcription factors than liver HS; thus, loss of these inhibitory functions in malignant tumors might promote the development of uncontrolled growth and gene expression favoring the neoplastic process. It is, however, noteworthy, that the profile of different PGs expressed is considerably modified when the hepatocyte becomes malignant [99]. It therefore remains to be shown to what extent the core protein influences these interactions.

It seems that the mitogenic activity of FGF-2 requires activation of nuclear targets. FGF-2 directly stimulates the activity of the casein kinase 2 (CK2), a ubiquitous serine/threonine kinase involved in the control of cell proliferation [100]. The function of CK2 as FGF-2 induced mitogenic effector might be, however, challenged by the presence of nuclear HS.

6.4. Regulation of cell proliferation, cell-cycle progression and migration

Pioneering work of Fedarko *et al.* shed light on the unique structure of nuclear HS [17]. Interestingly, both quantitative and structural changes accompany the growth inhibition of hepatocytes at confluence, as 3-fold increase of nuclear HS levels and a higher degree of sulfation were measured. Subsequently it was shown that exogenously added soluble HS oligosaccharides are able to enter the nucleus. A highly sulfated antiproliferative HS, rich in L-iduronic acid, could be detected in the nuclei of fibroblasts [22] and its presence was correlated with inhibition of cell proliferation. Observed differences in the uptake and nuclear localization of this anti-proliferative heparan sulfate between human lung fibroblasts and human lung carcinoma cells point toward a selectivity in uptake and internalization between benign and malignant cells. The lower sensitivity of tumor cells to the antiproliferative signal merits further investigations. HSPGs and growth factor receptors (GFRs) are often differentially expressed between benign and

malignant conditions. HSPGs act in a dual way both as internalization receptors and co-receptors for GFRs. Their expression, shedding and nuclear accumulation are highly regulated by a multitude of factors, which regulate their cell surface expression and thereby their availability, frequently in a tissue specific manner. This tumor and tissue specificity might explain the differences observed in various cell systems.

Recent data offer explanation for the anti-proliferative role of nuclear HS showing that HS inhibits the activity of histone acetyl-transferase p300, and pCAF [101]. This action is suspended by the nuclear heparanase [102]. Notably, xyloside priming modulates the biosynthesis and nuclear uptake of antiproliferative glycosaminoglycans concomitantly with attenuation of tumor growth and low acetylation of histone H3 [103], providing a mechanistic link for their functionality.

Mounting evidence suggests a correlation between nuclear HSPGs and cell cycle distribution. The nuclear FGF-2 and HS not only co-localize but also seem to regulate cell-cycle progression of various cells in a coordinated and dose-dependent manner. The nuclear entry of exogenous FGF-2 occurs around the G1 restriction point of the cell cycle [89]. Interestingly, exogenously added HS arrests cells transiently in the G1 phase [19] and decrease of nuclear HS level allows the regular cell cycle progression. Similarly, in the G1 phase, prominent nuclear glypican immune-reactivity is seen in glioma cells, indicating the decisive role of glypican in cell cycle progression [38]. Moreover, syndecan-1 accumulates in malignant mesothelioma cell nuclei by associating to tubulin structures during all phases of mitosis and inhibits their cell cycle progression, proliferation and directional migration [19,22,23,59,93,104]. The cell-cycle dependency of the nuclear entry of syndecan-1 could be demonstrated by interfering pharmacologically with tubulin structures [23], or by arresting cells in the G2/M phase of the cell cycle efficiently hampering the nuclear translocation of syndecan-1 [58]. TGF-β2 delays the nuclear translocation of syndecan-1 concomitantly with an anti-proliferative effect in malignant mesothelioma cells [105].

The various domains of syndecan-1 differ in their mechanism of action as evidenced by overexpression of full-length syndecan-1 and truncated versions in mesenchymal tumor cells. We could identify two distinct mechanisms responsible for decreased proliferation depending upon the functional domain of syndecan-1. While prolonged S phase was observed in full-length syndecan-1 transfectants, an increased length of G0/G1 phase was seen in the truncated versions containing only the cytoplasmic region or the RMKKK nuclear localization signal [84]. All syndecan-1 constructs hamper the directional movement of mesothelioma cells and the shortest RMKKK/EGFP construct has the most pronounced effect. It seems that the syndecan-1 effect on cell migration is not only dependent on its cell surface interaction, but also related to its presence in the nucleus. This effect may not only be a consequence of decreased cell migration but possibly also influenced by the reduced cell proliferation in these cells [84].

7. Potential for translational research

Heparan sulphate proteoglycans influence the behavior of malignant tumors in many different ways and syndecan-1 has been shown to fine-tune and coordinate many different signaling pathways [106]. Modulating their functions may offer powerful tools to control fundamental biological processes and provide the basis for subsequent targeted therapies in cancer. HSPG are efficient cargos and mediate internalization and nuclear delivery of a wide range of ligands. Such delivery offers promising possibilities of developing novel treatments of tumors [84,107–109]. The design of intracellular drug delivery vehicles, however, requires increased understanding of the precise molecular mechanisms that mediate cellular communication and transport across the plasma membrane and through the nuclear envelope.

Polyanionic carbohydrate moieties can block the enzymatic activity of heparanase and thereby interfere with the “heparanase/syndecan-1 axis”. In experimental systems these structures hamper tumor growth and some of them have now reached the early clinical trial stage. Another possibility to interfere with the synthesis of GAGs is to use aglycones that competitively hamper the glycanation of the protein core and/or the formation of free GAG chains. Experimentally such xylosides may inhibit tumor growth and angiogenesis [110,111].

The improved understanding of proteoglycan functions in malignancy indicates exciting possibilities to translate these findings into new possibilities to treat cancer. For example synstatin, a synthetic peptide containing the specific interacting motif of the syndecan-1 core protein, blocks growth of carcinomas and inhibits angiogenesis *in vivo* [112].

For such a translational perspective it will be important to know which of these reactions occur in the cell membrane and which relate to events inside the nucleus. Questions to be answered are: is the target available on the outside of the cell or the potential drug has to be taken up by the cell to have the desired effect? Although very promising, still much remains to be learned for sufficient understanding of the clinical potential [113,114].

Acknowledgments

The Hungarian Scientific Found (OTKA) 100904, the Swedish Heart and Lung Foundation, AFA Insurance, the Swedish Research Council and the Swedish Cancer Society have supported this review.

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