Review

Therapeutic value of glycosaminoglycans in cancer

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Abstract

Glycosaminoglycans are unbranched polysaccharides composed of repeating units of alternating uronic acids and amino sugars. Most glycosaminoglycans are covalently attached to core proteins to form proteoglycans. Posttranslational modifications result in specific motifs that bind to a large variety of ligands, thus regulating growth factor signaling, cellular behavior, inflammation, angiogenesis, and the proteolytic environment. Dysregulated expression of glycosaminoglycans is present in cancer and reported to correlate with clinical prognosis in several malignant neoplasms. Recent knowledge on the biological roles of these molecules in cancer biology, tumor angiogenesis, and metastasis has promoted the development of drugs targeting them. Pharmaceutical approaches include the use of chemically modified heparins and glycosaminoglycans with defined structures, combination of inhibitors of glycosaminoglycan biosynthesis and polyamine depletion, and biologically active glycosaminoglycan-binding peptides. In addition, glycosaminoglycans are used as tumor-specific delivery and targeting vehicles for toxins and chemotherapeutics. Encouraging results in animal studies and clinical trials show the clinical relevance of glycosaminoglycan-based drugs and the use of glycosaminoglycans as therapeutic targets. [Mol Cancer Ther 2006;5(9):2139–48]

An Introduction to Glycosaminoglycans and Proteoglycans

Glycosaminoglycans are long, unbranched polysaccharides composed of repeating disaccharide units consisting of alternating uronic acids and amino sugars (ref. 1; Fig. 1A). Four major classes of glycosaminoglycans have been identified, all of which have relevance in cancer: heparan sulfate, chondroitin sulfate/dermatan sulfate, keratan sulfate, and hyaluronan. Posttranslational modifications such as epimerization and sulfation result in structural diversity and formation of specific binding motifs for many ligands (1, 2). Hyaluronan is the only glycosaminoglycan without sulfate groups. Physiologically, most glycosaminoglycans are covalently attached to core proteins to form proteoglycans. Proteoglycans are classified based on the amino acid homology of their protein cores, their location [cell surface, basement membrane, or extracellular matrix (ECM)], and their glycosaminoglycan substitution (1, 2). However, some proteoglycans are substituted with more than one glycosaminoglycan chain type, such as syndecan-1 (heparan sulfate and chondroitin sulfate) and aggrecan (keratan sulfate and chondroitin sulfate; refs. 1, 2). In this review, the biological roles and therapeutic values of hyaluronan, together with selected examples of cell-surface and matrix heparan sulfate and small leucine-rich proteoglycans, in cancer are discussed. In addition, cancer-related functions of glycosaminoglycan receptors and enzymes involved in glycosaminoglycan synthesis and modification are presented.

Roles of Glycosaminoglycans and Proteoglycans in Cancer

Glycosaminoglycans and proteoglycans both play major roles in multiple cancer-related processes. Changes in expression of these molecules, as well as of enzymes involved in their biosynthesis and degradation, contribute to the different steps of tumor progression. Due to space limitations, we will use selected examples to show the diverse roles of glycosaminoglycans and proteoglycans in cancer. The reader is referred to a number of recent reviews for a more comprehensive view (3–7).

Cancer Cell Proliferation and Growth

Rapid cell proliferation is an important characteristic of malignant transformation. There is ample evidence for a role of glycosaminoglycans and proteoglycans in controlling cell proliferation. Cell-surface heparan sulfate proteoglycans serve as coreceptors for several growth factor tyrosine kinase receptors, which transduce signals on formation of a ternary complex of ligand, receptor, and heparan sulfate proteoglycan (ref. 2; Fig. 1B). In some cases, heparan sulfate proteoglycan–bound growth factors are released by heparanase, a β-endoglucuronidase that cleaves glycosidic bonds in heparan sulfate via hydrolysis, to achieve an activating

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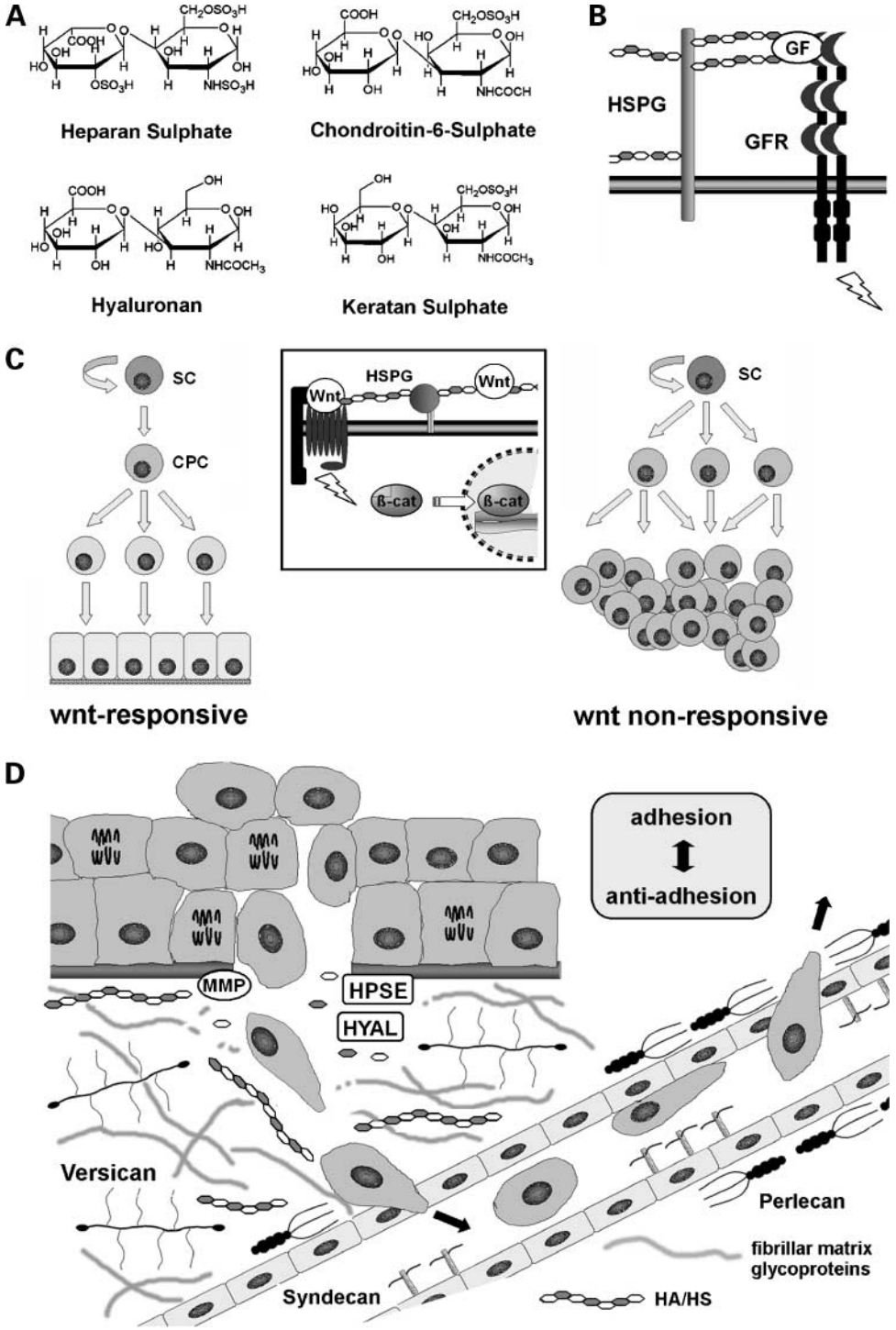
effect (2, 3). Chondroitin sulfate proteoglycans/dermatan sulfate proteoglycans have also been shown to have a role as modulators of signal transduction. Melanoma chondroitin sulfate proteoglycan enhances focal adhesion kinase and extracellular signal–regulated kinase activation (8), and the dermatan sulfate proteoglycan decorin modulates epidermal growth factor receptor signaling, thus controlling cell proliferation (9). Overexpression of hyaluronan synthase 2 increases ErbB2-dependent signaling in breast cancer cells (10), whereas antisense-mediated suppression of hyaluronan synthase 2 inhibits tumorigenesis and progression of breast cancer (11).



Invasion and Metastasis of Cancer Cells

The ability of cancer cells to invade into surrounding tissues involves changes in expression of cell-surface molecules and the expression of ECM-degradative enzymes (refs. 3, 4, 12; Fig. 1D). Glycosaminoglycans and proteoglycans are major constituents of the ECM and cell-surface proteoglycans mediate cell-matrix interactions. Changes in expression of these molecules reduce cell adhesion and promote cancer cell invasion. For example, versican, produced by prostate cancer cells, inhibits cell adhesion to fibronectin (13). Syndecans, acting in concert with integrins (2), and hyaluronan, signaling through CD44 (4), contribute to increased cancer cell motility through signaling events that activate the cytoskeleton. Hyaluronan production is related to the metastatic potential of mouse mammary carcinoma cells (14). Cancer cells also secrete matrix metalloproteinases, heparanase, and hyaluronidases to penetrate the basement membrane and ECM to invade surrounding tissues (3, 4, 12, 15).

Metastasis depends on cancer cell dissemination into the circulation and adhesive interactions with endothelial cells, leukocytes, and platelets, ultimately resulting in their colonization of distant tissues and organs (refs. 3, 4, 12, 15; Fig. 1D). Heparanase promotes invasion and metastasis by degrading heparan sulfate chains in cell-surface and matrix heparan sulfate proteoglycans (16–19). Loss of syndecan-1 *in vivo* results in increased leukocyte-endothelial interactions (20). Syndecan-1 may regulate the adhesion of cancer cells to blood and lymphatic vessel endothelium or promote the association with different host cells during metastatic seeding. The antimetastatic action of heparin has, at least partly, been ascribed to its interference with endothelial P-selectin *in vivo* (21).

Angiogenesis

For a cancer to grow beyond a diameter of 2 mm, primary tumors and metastases require nutrient support from the vascular system. Thus, angiogenesis is a crucial process that is targeted in cancer therapy (18). Apart from growth factors such as vascular endothelial growth factors, fibroblast growth factors, and angiopoietins, glycosaminoglycans and proteoglycans are involved in angiogenesis (3, 6, 20, 22–24). We recently showed increased angiogenesis in syndecan-1-deficient mice (20) and the formation of abnormally dilated blood vessels in syndecan-1-overexpressing mice (23). Heparanase stimulates angiogenesis via angiogenic factor mobilization and induction of cyclooxygenase-2 and vascular endothelial growth factor (25). Antisense inhibition of perlecan led to decreased colon carcinoma growth and tumor angiogenesis, and similar findings were obtained in perlecan-deficient mice (6, 26, 27). Chondroitin sulfate exerts antiangiogenic effects via inhibition of transendothelial monocyte migration (28). Moreover, decorin suppresses tumor angiogenesis through down-regulation of vascular endothelial growth factor production by cancer cells (24).

Cancer Stem Cells

Cancer stem cells have recently attracted considerable interest among scientists and oncologists. They have been identified in a range of cancers and are proposed to represent the cells of origin of these tumors (29). Due to their long life span, drug resistance through expression of ABC transporters, active DNA repair capacity, and apoptosis resistance, cancer stem cells may represent a subpopulation of tumor cells particularly resistant to chemotherapy. Glycosaminoglycans and proteoglycans have been identified as part of specific marker signatures of progenitor cells. For example, the melanoma chondroitin sulfate proteoglycan marks a class of epidermal stem cells (30), the chondroitin sulfate proteoglycan NG2 marks oligodendrocyte progenitors (31), and the 473HD-chondroitin sulfate epitope marks multipotent progenitor cells of the developing telencephalon (32). Glycosaminoglycans and proteoglycans play major supportive roles in developmental signaling (3) and provide a niche for preservation of cell ''stemness.'' This has been shown for chondroitin sulfate proteoglycan in neural stem cells (33) and for heparan sulfate proteoglycan and chondroitin sulfate proteoglycan in hematopoietic precursor cells (34, 35).

It has been proposed that reduction in NG2 expression may be part of a switch between proliferation and migration in primitive, stem cell–derived neuroectodermal tumors (31). In addition, syndecan-1-deficient mice are largely resistant to mammary tumor formation (3). This

Figure 1. Structure and cancer-related functions of glycosaminoglycans. **A,** glycosaminoglycan disaccharide units. Heparan sulfate: *N*-acetyl-glucosamine-α-L-iduronic acid/β-D-glucuronic acid; heparin displays a higher degree of sulfation and iduronic acid content compared with heparan sulfate; chondroitin sulfate; *N*-acetyl-β-D-galactosamine-D-glucuronic acid. Dermatan sulfate is derived from chondroitin sulfate by C5-epimerization of the β-D-glucuronic acid residue. Keratan sulfate: *N*-acetyl-β-D-glucosamine-β-D-galactose. Hyaluronan: *N*-acetyl-β-D-glucosamine-D-glucuronic acid. **B,** heparan sulfate proteoglycans act as coreceptors for growth factor receptor (*GFR*) signaling, thus promoting cancer cell proliferation and angiogenesis. **C,** cancer stem cells. Omnipotent, slow cycling stem cells generate a pool of rapidly cycling pluripotent committed progenitor cells, which ultimately become mature terminally differentiated cells (*left*). In cancer, progenitor cell proliferation is no longer restricted, leading to massive clonal expansion and lack of differentiation (*right*). The wnt signaling pathway, which is modulated by glypican family of heparan sulfate proteoglycan (*middle*), stimulates stem cell/commited progenitor cell proliferation. Although the mode of glypican action is not fully understood, wnt signaling may be promoted by presentation of heparan sulfate proteoglycan – bound wnt to its receptor or by increasing the concentration of wnt at the cell surface via heparan sulfate proteoglycan-wnt interactions. **D,** cancer cell invasion and metastasis (see text). Malignant cells need to loosen cell-cell and cell-matrix contact to invade the surrounding tissues and need to regain adhesiveness on escape from the circulation. The balance of adhesion and antiadhesion is modulated by glycosaminoglycans and proteoglycans. Glycosaminoglycans and proteoglycans in the basement membranes of epithelia and endothelia and ECM are degraded by matrix metalloproteinases (*MMP*), heparanase (*HPSE*), and hyaluronidases (*HYAL*). Cell adhesion and migration are modulated by glycosaminoglycans and proteoglycans such as versican, CD44/hyaluronan, and syndecans. To escape from the circulation, disseminated tumor cells aggregate with leukocytes and platelets and adhere to the vessel wall, a process involving heparan sulfate (*HS*) proteoglycans and chondroitin sulfate proteoglycans via direct interactions with and modulation of chemokine signaling. These functions also contribute to tumor angiogenesis (cf. **B**).

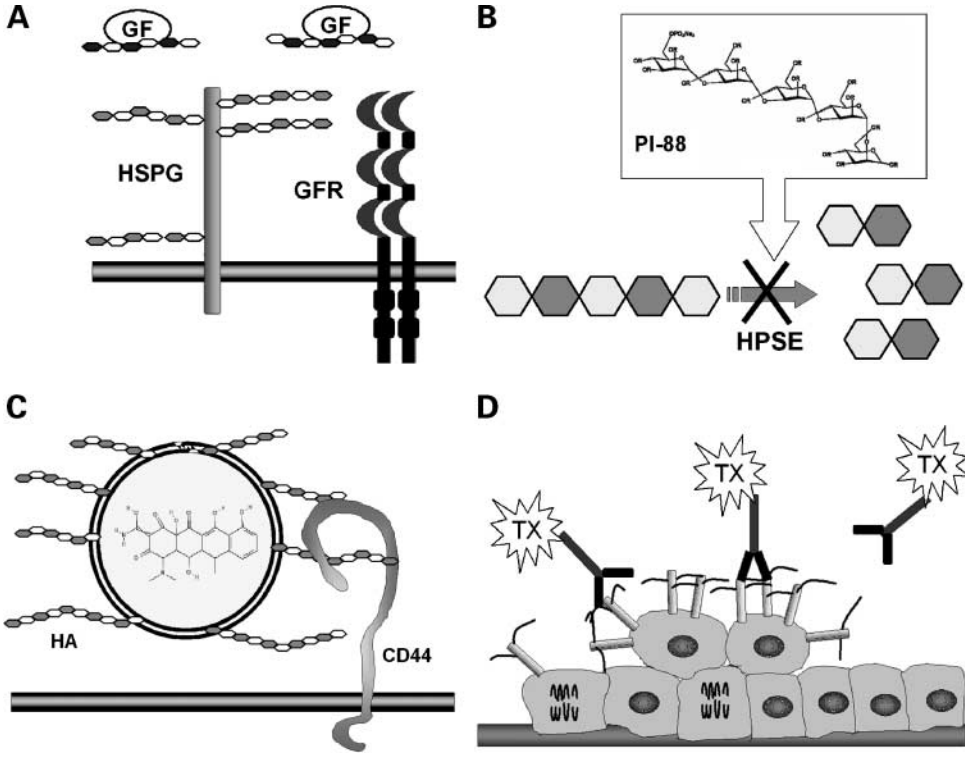


Figure 2. Selected therapeutic applications of glycosaminoglycans (see text for details). **A,** competitive binding of (modified) heparins and mimetic glycosaminoglycans to growth factors (*GF*) reduces cancer cell proliferation and angiogenesis (cf. Fig. 1B). **B,** inhibition of heparanase activity by the substrate analog PI-88 prevents heparan sulfate degradation and reduces tumor cell invasion, metastasis, and angiogenesis. **C,** incorporation of HA into doxycycline-loaded liposomes leads to more specific delivery to CD44-overexpressing cancer cells and to CD44-mediated internalization. **D,** toxin (*TX*)-coupled antibodies selectively target proteoglycans highly expressed by cancer cells.

could be attributed to a decreased wnt-1-responsive progenitor cell population in syndecan-1-deficient mammary glands (ref. 36; Fig. 1C). Of note, it has recently been shown in a mouse model that embryonic stem cell–derived dendritic cells engineered to express glypican-3 confer protective immunity against highly metastatic, glypican-3-expressing B16-F10 melanoma cells (37).

An additional antitumor strategy is the induction of terminal differentiation in cancer cells (38). Heparan sulfate proteoglycan expression is associated with differentiation of progenitor cells and protects against apoptosis, as shown in patient cells deficient in the heparan sulfate copolymerases EXT1/EXT2 (39) or the glycosaminoglycan-catabolizing enzyme α-L-iduronidase (40). Because nuclear targeting of heparanase induces differentiation of human breast cancer cells (38), a targeted modulation of glycosaminoglycan/proteoglycan expression in cancer stem cells may be a promising approach, leading to either apoptosis or loss of the malignant properties of the cells.

Glycosaminoglycans and Proteoglycans as Diagnostic and Prognostic Factors

Dysregulated expression of glycosaminoglycans and proteoglycans, as well as of enzymes involved in their biosynthesis and degradation, has been reported to affect all stages of tumorigenesis. Of note, a prognostic value for the clinical outcome of cancer has recently been assigned to changes in expression of several glycosaminoglycans and proteoglycans. These findings have raised considerable interest in the generation of glycosaminoglycan/proteoglycan–based diagnostic tools. In addition to more traditional molecular biology and immunohistochemical approaches, the recent development of highly sensitive mass spectrometry techniques has facilitated structural and sequence analysis of glycosaminoglycans, even in minute amounts of tissue samples (5, 41–43). These methods were used to identify tumor growth–promoting and tumor growth–inhibiting sequences in heparan sulfate (44) and to detect decorin, biglycan, perlecan, versican, syndecan-1, and syndecan-4 in colon cancer, pancreatic cancer, and fibrosarcoma tissue (45–47). In the succeeding sections and in Supplementary Table S1[3] online, examples illustrating the prognostic values of glycosaminoglycans and proteoglycans in cancer are discussed.

Heparan Sulfate

Heparan sulfate undergoes specific structural changes during the progression of human colon adenoma to carcinoma. Colon carcinoma cells have a 33% reduction in 2-O-sulfation on iduronic acid and a 20% reduction in overall N-sulfation compared with adenoma cells (48). These changes strongly influence binding of ligands and alter the biological functions of the affected heparan sulfate proteoglycan (2). Consequently, prognostic and predictive values could be assigned to qualitative and quantitative changes in heparan sulfate expression (48, 49).

[3] Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

Cell-Surface Heparan Sulfate Proteoglycans

The majority of cell-surface heparan sulfate is found in two families of membrane-bound proteoglycans, the transmembrane-anchored syndecans and the glycosylphosphatidylinositol-anchored glypicans. Via their heparan sulfate chains, syndecans and glypicans bind a large variety of extracellular ligands, thus modulating morphogenesis and wound repair, inflammation, host defense, and energy metabolism (2). Although several family members seem to be involved in the pathogenesis of cancer, most of the published data has focused on two model members, syndecan-1 and glypican-3.

Syndecan-1 is a prognostic marker for several cancer types (Supplementary Table S1).[3] It contributes to cell proliferation as a coreceptor for several growth factor receptors and acts as a cell adhesion molecule and modulator of proteolysis, chemokine action, angiogenesis, and stem cell function (2, 20, 23, 36).

Glypican-3, a lipid-anchored membrane heparan sulfate proteoglycan, has been proposed to act as a tumor suppressor in some cancers. It is epigenetically silenced by promoter hypermethylation in breast cancer. Mutations in the *GPC3* gene lead to the Simpson-Golabi-Behmel syndrome (7, 50). However, its role as a tumor suppressor does not apply to all cancer types and may depend on the cellular context.

Heparan Sulfate Synthesis and Modification Enzymes: EXT1, EXT2, and HSulf-1

Mutations in *EXT1* and *EXT2*, which encode heparan sulfate copolymerases, are linked to hereditary multiple exostoses and malignant chondrosarcomas (51). The enzyme HSulf-1 modifies heparan sulfate sulfation and is markedly diminished in ovarian cancer, hepatocellular carcinoma, and head and neck squamous cell carcinoma (3, 52). In contrast, HSulf-1 expression is increased in pancreatic cancer (53). Sulfation is a critical determinant of ligand binding to heparan sulfate and affects multiple processes relevant to cancer progression. Indeed, expression of both HSulf-1 and HSulf-2 in transfected human myeloma cells leads to dramatically decreased tumor growth *in vivo* accompanied by reduced formation of the ternary fibroblast growth factor-2 signaling complex and increased matrix deposition (54).

Heparanase

Heparanase promotes metastasis and modulates cell proliferation and angiogenesis via mobilization of angiogenic and growth factors from heparan sulfate proteoglycan (15, 25). In animal models, silencing of heparanase in lymphoma, melanoma, and breast cancer cells leads to improved survival and reduced metastasis and tumor angiogenesis (55). Heparanase is an important prognostic factor in a number of neoplasms, including ovarian, breast, colon, cervical, pancreatic, gastric, esophageal, and head and neck cancers (16, 17, 56–62).

Chondroitin Sulfate

Versican, a large aggregating chondroitin sulfate proteoglycan, is located primarily within the ECM and shows increased expression in several cancers (Supplementary Table S1).[3] It interacts with a multitude of binding partners, including hyaluronan, CD44, type I collagen, tenascin-R, fibulins, fibronectin, selectins, chemokines, and epidermal growth factor receptor (63, 64).

Small Leucine-Rich Proteoglycans

The secreted dermatan sulfate proteoglycan decorin modulates growth factor receptor activities and availability, cell adhesion, and angiogenesis (9, 24, 65). Its role in cancer is context dependent (Supplementary Table S1).[3] The keratan sulfate proteoglycan lumican inhibits melanoma progression (66), whereas the dermatan sulfate proteoglycan biglycan is part of an expression signature characterizing chemoresistant osteosarcoma (67). Biglycan-binding proteins are up-regulated in malignant cell lines relative to benign cells (65).

Hyaluronan, Hyaluronan Synthases, Hyaluronan Receptors, and Hyaluronidase

A role in cancer metastasis has been established for hyaluronan (4, 68). Hyaluronan receptors, hyaluronan synthases, and hyaluronidase regulate extracellular hyaluronan concentration and/or signal on hyaluronan binding. Expression changes in hyaluronan and its receptors (receptor for hyaluronan-mediated motility, CD44 isoforms, and LYVE-1) are of prognostic value in several cancers (ref. 4; Supplementary Table S1).[3] Using antisense cDNA transfection methods, a role for hyaluronidase-1 in tumor growth and invasion was established for bladder cancer in a xenograft model (68). In non-Hodgkin's lymphoma, hyaluronan and hyaluronidase-2 expression correlated with lymphoma subtype (69). Importantly, hyaluronan was overexpressed in aggressive subtypes whereas hyaluronidase-2 expression was down-regulated. A diagnostic value for hyaluronan synthase expression has been shown in multiple myeloma, ovarian cancer, endometrial cancer, and colon cancer (14, 70).

Glycosaminoglycan- and Proteoglycan-Based Approaches of Cancer Therapy

Chemically Modified Heparins and Glycosaminoglycans

Heparin is a potent anticoagulant used for decades to prevent and treat thromboembolism. In cancer, its anticoagulant activity affects tumor progression by decreasing thrombin generation and fibrin formation (71). Several animal studies suggest that its antimetastatic activity is based on its anticoagulant activity, inhibition of heparanase, interference with P-selectin-heparan sulfate proteoglycan interactions, and inhibitory effect on tumor cell adhesion and motility (refs. 3, 20, 71, 72; Fig. 2A).

As early as 1984, Drago et al. (73) demonstrated that heparin and heparan sulfate reduced cancer metastasis in the Nb rat prostatic adenocarcinoma model. The low molecular weight heparin reviparin not only inhibited collagen adhesion and Matrigel invasion of adenocarcinoma cells but also reduced their intraabdominal growth *in vivo* (74). Although the anticoagulant activity may contribute to the antitumoral properties of heparin, anticoagulation constitutes a potential adverse effect in cancer therapy. Thus,

syntheses and studies of antitumoral activity of heparins and heparinoids with low anticoagulant activity have gained a lot of attention. Kragh et al. (75) examined the antimetastatic activity of the low molecular weight heparin tinzaparin and several non-anticoagulant heparin derivatives in the syngeneic B16-F10 melanoma mouse model of metastasis. A non-anticoagulant heparin derivative of 8 kDa reduced metastasis by 58% but did not affect primary cancer growth (75). Low anticoagulant heparin was found to be as effective as heparin in inhibition of lung colonization by Lewis lung carcinoma cells (76). In a randomized clinical trial, Klerk et al. (77) studied the effect of the low molecular weight heparin nadroparin on survival of 302 patients with advanced malignancy without venous thromboembolism and reported that heparin treatment favorably influenced survival. In the Fragmin Advanced Malignancy Outcome Study, 385 patients with advanced malignancy were randomly assigned to receive either single daily s.c. injections of the low molecular weight heparin deltaparin or placebo for 1 year (78). Although deltaparin treatment did not substantially improve 1-year survival in the cancer patients, substantially improved survival was noted in a subgroup of patients with a better prognosis. Thus, it may be necessary to define patient groups in which heparin treatment could prolong survival (78). Several studies suggest that a combination of conventional chemotherapy with heparin treatment may be the way to go. In a randomized clinical trial of small-cell lung cancer patients, combined treatment with low molecular weight heparin and cyclophosphamide, epirubicine, and vincristine led to significant improvements in tumor response rates, median progression-free survival, and median overall survival compared with chemotherapy alone (79). These encouraging initial results need to be confirmed in further clinical trials.

Besides heparin, heparin analogues and mimetics, as well as modified heparan sulfate and chondroitin sulfate, have been studied as potential cancer therapeutics. In an attempt to generate a potentially therapeutic mimetic of syndecan-1, Pumphrey et al. (80) discovered that carbodiimide-modified glycosaminoglycans reduced breast cancer and myeloma cell viability by inducing apoptosis. Moreover, modified chondroitin sulfate abolished breast tumor growth in nude mice. Heparan sulfate mimetics with anticancer properties such as KI-111 [2-(4-fluoro-3-nitrobenzoyl)benzoic acetic anhydride] inhibited tumor cell adhesion, migration, growth, and invasion *in vitro*. In contrast, other KI compounds inhibited cancer invasion and migration but promoted tumor cell adhesion (81, 82).

Inhibitors of Glycosaminoglycan Biosynthesis

Given the importance of glycosaminoglycans in cancer, one therapeutic approach is inhibition of glycosaminoglycan biosynthesis. The antimetastatic reagent 5-hexyl-2-deoxyuridine reduces biosynthesis of heparan sulfate and other glycoconjugates by inhibiting the conversion of glucosamine to UDP-sugars (83). Furthermore, cells depend on nucleic acid–binding polyamines for growth, as they are essential for a variety of DNA-related functions, including replication and transcription. Belting et al. (84) tested the hypothesis that heparan sulfate proteoglycans could be involved in a salvage pathway for uptake of circulating polyamines, a potential mechanism of escaping polyamine-depletion anticancer therapy. *In vitro*, mutant Chinese hamster ovary cells deficient in heparan sulfate biosynthesis were more susceptible to α-difluoromethylornithine–mediated polyamine depletion than wild-type cells. In a mouse metastasis model, α-difluoromethylornithine reduced seeding and growth of tumor foci in the lungs by heparan sulfate–deficient mutant cells.

Hyaluronan biosynthesis in murine melanoma cells can be effectively inhibited by 4-methylumbelliferone without cytotoxic effects (85). Of note, cancer cell adhesion and invasion were dose-dependently inhibited by 4-methylumbelliferone. In a syngeneic mouse metastasis model, 4-methylumbelliferone pretreatment of melanoma cells led to reduced cell-surface hyaluronan formation and suppression of liver metastases (86). Thus, 4-methylumbelliferone seems to be a good candidate for an antimetastatic agent in tumors with dysregulated hyaluronan synthesis.

Inhibitors of Glycosaminoglycan-Degrading Enzymes

Because dysregulated glycosaminoglycan degradation is mechanistically important in cancer, targeting glycosaminoglycan-degrading enzymes is a logical anticancer strategy. Heparanase is implicated in several steps of tumor progression, as recently shown in stage-specific pharmacologic trials on the RIP-Tag2 mouse model of pancreatic cancer (61). Heparanase expression increased progressively through multiple stages of tumorigenesis. Importantly, the sulfated oligosaccharide phosphomannopentose sulfate (PI-88; Fig. 2B) reduced early progenitor lesions and inhibited cancer growth at late stages. PI-88 is a structural mimetic and inhibits both heparanase activity and heparan sulfate effector functions, resulting in decreased cancer cell proliferation and angiogenesis and increased apoptosis in the RIP-Tag2 mice. The antiangiogenic activity of PI-88 is comparable to that of endostatin (87). Currently, PI-88 is in phase II clinical trials (82). The β-1,3-sulfated glycan laminarin, but not its unsulfated form, inhibits heparanase activity (88). In rodent models, laminarin sulfate reduced the extent of lung colonization with i.v. injected mouse melanoma and rat mammary carcinoma cells by 80% to 90%.

Attempts have also been made to transform heparan sulfate/heparin into a heparanase inhibitor by selective chemical modification. Glycol-split *N*-acetyl heparins are potent inhibitors of heparanase and do not release fibroblast growth factor-2 from ECM, thus mediating potential antimetastatic and antiangiogenic effects (89). Freeman et al. (90) synthesized heparan sulfate mimetics as a tool to probe the heparan sulfate binding specificity of several heparan sulfate ligands. Of note, heparanase activity was most effectively blocked by heparan sulfate mimetics resembling a sulfated pentasaccharide (90). Ishida et al. (81) compared a database of 50,000 compounds to the structure of the heparan sulfate disaccharide unit HexUA-GlcNAc(6S) to develop heparanase-inhibiting heparan

sulfate mimetics. Among several 2-(3-nitrobenzoyl)benzoic acid derivatives, KI-105 inhibited migration and invasion of human HT-1080 fibrosarcoma cells although it was only a moderate inhibitor of heparanase activity. Suramin, a polysulfonated naphthylurea, and its derivatives have also been used as heparanase inhibitors. Suramin inhibits the binding of several growth factors to their receptors and interferes with glycosaminoglycan catabolism, resulting in reduced cancer cell proliferation and angiogenesis (91). At least some of the antineoplastic properties of suramin seem to be based on heparanase inhibition. Due to the toxicity of suramin, less toxic suramin analogues of equal or higher antitumoral potency have recently been developed (92).

Biologically Active Glycosaminoglycan-Binding Peptides

Another therapeutic strategy for interfering with glycosaminoglycan function is represented by the use of inhibitory glycosaminoglycan-binding peptides. The relevance of this approach in anticancer therapy has recently been shown *in vitro* and *in vivo*. A peptide (P4), with strong binding to hyaluronan, inhibited cell growth in culture and in chorioallantoic membrane assays and reduced vascular endothelial growth factor–mediated angiogenesis. On vector-based expression in cancer cells, P4 reduced growth and vascularization in a nude mouse model (93). A similar approach could be applied to other glycosaminoglycans. For example, peptides containing concatameric consensus sequences of heparin-binding proteins exhibit high binding affinities for both heparin and endothelial cell heparan sulfate proteoglycan (94). Thrombospondin-1-derived heparin-binding peptides induced apoptosis in promyelocytic leukemia cells (95).

Glycosaminoglycans as Tumor-Specific Targeting Vehicles for Toxins and Chemotherapeutics

An important supportive role can be assigned to some glycosaminoglycans, which serve as targeting vehicles for delivery of toxins and chemotherapeutics to cancerous tissues. The most extensively used glycosaminoglycan in this context is hyaluronan. Hyaluronan is efficiently internalized by a variety of cells via its receptors receptor for hyaluronan-mediated motility, CD44-isoforms, LYVE-1, and HARE (4). Because many tumors overexpress these receptors, the coupling of cytotoxic drugs to hyaluronan is a promising strategy. The nontoxic prodrug is activated on endocytosis of hyaluronan, reducing side effects of the therapy and increasing cancer cell specificity (96). In some instances, hyaluronan has been directly coupled to anticancer drugs and toxins (97, 98). Butyric acid, an inhibitor of histone deacetylase, retains its inhibitory activity when coupled to hyaluronan by esterification. In studies on syngeneic mice, primary tumor growth and lung and liver metastases were drastically reduced by hyaluronan-butyric acid treatment, resulting in prolonged survival (98). Hyaluronan has also been incorporated into liposomes for tumor-targeting purposes (ref. 99; Fig. 2C). Doxycycline-hyaluronan liposomes showed almost 10 times higher cytotoxicity compared with the drug alone and more than 100 times higher activity than liposomes devoid of hyaluronan. These *in vitro* studies were recently confirmed and extended in syngeneic and human xenograft mouse models (100).

Glycosaminoglycans and Proteoglycans as Tumor-Specific Targets for Toxin Delivery

Syndecan-1 is a tumor marker for a number of cancers (Supplementary Table S1).[3] This property was recently exploited using syndecan-1 as a target for antibody-mediated toxin-delivery to cancer cells (ref. 101; Fig. 2D). The antimicrotubule agent DM1 was coupled to the monoclonal antihuman syndecan-1 antibody B-B4. The immunoconjugate was effective *in vitro*, selectively decreasing growth and survival of multiple myeloma cell lines, patient multiple myeloma cells, and multiple myeloma cells adherent to bone marrow stromal cells. In severe combined immunodeficient (SCID) mice, xenograft models and human fetal bone transplants bearing patient multiple myeloma cells, B-B4-DM1 treatment resulted in tumor growth inhibition and regression and improvement in overall survival. Whereas these results were encouraging, a major drawback of the study is that some toxic side effects of B-B4-DM1 could not be tested in the SCID mouse model. In a human host setting, B-B4-DM1 could react with tissues of epithelial origin and potentially cause side effects. Nevertheless, short-term treatment or combined treatment with conventional chemotherapy may be therapeutically beneficial.

Chondroitin sulfate is also a molecular target for chemotherapy delivery. Liposomes containing the cationic lipid 3,5-dipentadecycloxybenzamidine hydrochloride (TRX-20) display preferential binding to chondroitin sulfate. Delivery of cisplatin to chondroitin sulfate–expressing cancer cells via TRX-20 liposomes was more efficient than delivery via plain liposomes both *in vitro* and *in vivo* (102, 103).

Glycosaminoglycans and Proteoglycans as Therapeutics

In some cases, proteoglycans per se, such as decorin, seem to be effective potential therapeutics. In an orthotopic mammary carcinoma model, treatment with decorin core protein reduced primary tumor growth by 70% and eliminated metastases (9). Decorin seems to be involved in regulation of epidermal growth factor receptor signaling and endocytosis-mediated receptor down-regulation (9, 65). Adenovirus-mediated decorin delivery resulted in comparable effects (9). It was also effective in inhibiting growth of tumors in a nude mouse xenograft model, leading to overexpression of p21WAF1, an inhibitor of cyclin-dependent kinase activity (104). In a separate xenograft study employing decorin-transfected cancer cell lines, ectopic decorin expression reduced cancer growth rate and angiogenesis (24). In a rat glioma model, ectopic expression of decorin in CNS-1 cells resulted in significantly increased survival of animals bearing decorin-transfected cells in comparison with control cells (105). Based on these data, the application of decorin in a clinical setting may be a promising approach. However, direct administration of decorin faces the problem of molecular heterogeneity caused by structural and size variability of the glycosaminoglycan chain. This constitutes a technical

challenge with regard to biotechnological production of decorin of a consistent chemical composition.

Glycosaminoglycans and Proteoglycans as Angiogenesis Inhibitors

Pharmacologic inhibition of angiogenesis is a well-established approach in cancer therapy (6, 22). Many classes of glycosaminoglycans and proteoglycans participate in angiogenesis, including heparan sulfate, chondroitin sulfate, and matrix and cell-surface proteoglycans. Among the glycosaminoglycan-based pharmaceutics already in clinical use, modulation of angiogenesis by different heparins has been shown. For example, the low molecular weight heparin tinzaparin was found to be a potent angiogenesis inhibitor *in vitro* (106) and heparin octasaccharides inhibited tumor angiogenesis in animal models (107). Mechanistically, this antiangiogenic effect is due to heparin-induced cellular release of tissue factor pathway inhibitor. Whereas the results of these preclinical studies are encouraging, in most cases the anticoagulant properties of heparin represent an undesirable side effect in cancer therapy. Thus, as previously discussed, an alternative approach is the development and use of heparin species and derivatives with low anticoagulant activity that could act as competitive inhibitors of angiogenic factors such as fibroblast growth factor-2 and vascular endothelial growth factor (Fig. 2A). Heparin-based antiangiogenic therapies may also be the method of choice in cases where tumor angiogenesis depends on cell-surface heparan sulfate proteoglycans (3, 20, 23). Heparanase, hyaluronan, and decorin are also implicated in angiogenesis (4, 15, 24, 87) and therapeutic strategies targeting these molecules have been discussed above.

Conclusions

Glycosaminoglycans and proteoglycans are involved in the pathobiology of all stages of cancer progression. Importantly, changes in expression of glycosaminoglycans and proteoglycans have diagnostic and prognostic values in several cancers and may increasingly become valuable in planning of targeted cancer therapies. Development of novel diagnostic tools such as glycomic-based glycoprofiling array techniques will facilitate detailed analysis and will complement classic ELISA and antibody-based histopathologic methods. Furthermore, improvements in analytic and glycosaminoglycan sequencing techniques will increase our knowledge on the roles of specific glycosaminoglycan epitopes in cancer. In therapeutics, targeting of glycosaminoglycans and proteoglycans and the use of glycosaminoglycans, proteoglycans, and their mimetics are highly promising. Due to the multifaceted functions of glycosaminoglycans and proteoglycans in cancer pathobiology, interference with their function frequently results in inhibition of the malignant process at multiple stages. However, the diverse functions of glycosaminoglycans and proteoglycans necessitate careful, context-dependent therapeutic application. For example, a glycosaminoglycan species may inhibit cancer cell attachment and prevent distant metastatic seeding of circulating tumor cells. However, it may also promote escape of tumor cells from the primary tumor. Moreover, the anticoagulant function of heparin species helps to prevent formation of an immunoprotective fibrin coat around tumor cells, but may cause bleeding problems in cancer patients. Progress in development of glycosaminoglycan/proteoglycan mimetics that act only on specific steps of tumor progression will allow for more selective therapy. Use of these approaches combined with conventional chemotherapy has already shown synergistic effects in cancer treatment.

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