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Proteoglycans on bone tumor development

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Proteoglycans, extracellular matrix components, exert several activities on bone cells and seem crucial for maintaining an appropriate number of osteoblasts and osteoclasts. The overall data strengthen a pro-bone resorptive role for proteoglycans, through the control of osteoprotegerin availability and of receptor activator of NF-κB ligand bioactivity. In parallel, proteoglycans participate in the control of tumor development at different levels, including bone tumor development and bone metastases dissemination. This dual role makes them good candidates as regulatory molecules in the vicious cycle between tumor proliferation and bone resorption observed during tumor development in bone site. Knowledge of the biological roles of these molecules in cancer biology, tumor angiogenesis and metastasis has promoted the development of drugs targeting them.

Introduction

Proteoglycans (PGs) are composed of a core protein with covalently attached glycosaminoglycan (GAG) chains [1]. The GAGs are linear polymers of repeated disaccharidic units of hexosamine and hexuronic acid (except for keratan sulfate, in which hexuronic acid is replaced by galactose). These GAGs are sulfated, and the degree and position of sulfate are extremely variable in sulfated GAG, depending on the tissular and/or cellular and/or metabolic context, ensuring structural variability of these polysaccharides [2] (Table 1).

These PGs are ubiquitous, being present as cell surface molecules anchored in the plasma membrane, as components of the extracellular matrix (ECM) or as soluble molecules present in ECM and serum (Table 2). Soluble PGs, as well as those bound within the ECM, are derived from cell secretions or by shedding from the cell surface. PGs function in both cell–cell and cell–ECM adhesion and can also act to promote assembly of ECM molecules [2]. In addition, PGs bind a wide range of bioactive molecules that regulate cell behaviors in normal and pathological processes. Thus, PGs or GAGs associated to the cell membrane or resident in the extracellular bone matrix regulated bone growth and remodeling [3].

Bone is continually remodeled according to physiological events. This remodeling results from the activities of many cell lineages. Their cellular interactions control their cellular activities and the bone remodeling intensity. These interactions can be established either through cell–cell contact or by the release of many polypeptidic factors and/or their soluble receptor chains. These factors can act directly on osteogenic cells and their precursors to control differentiation, formation and functions. Thus, osteoprotegerin (OPG)/receptor activator of NF-κB (RANK)/RANK ligand (RANKL) have been identified as members of a ligand–receptor system that directly regulates osteoclast differentiation and osteolysis. Whereas RANKL is a powerful inducer of bone resorption through its interaction with RANK, OPG is a soluble decoy receptor and acts as a strong inhibitor of osteoclastic differentiation. Any dysregulation of their respective expression leads to pathological conditions. Furthermore, recent data demonstrate that the OPG/RANK/RANKL system modulates cancer cell migration, thus controlling the development of bone metastases [4].

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TABLE 1

Composition of glycosaminoglycans

Glycosaminoglycan	Repeating disaccharide	Sulfation position
Hyaluronan	D-Glucuronic acid and N-acetylglucosamine	No sulfation
Chondroitin sulfate	D-Glucuronic acid and N-acetylgalactosamine	GalNAc-4 SO_4 GalNAc-6 SO_4 GalNAc-4,6 SO_4
Dermatan sulfate	D-Glucuronic acid/L-iduronic acid and N-acetylgalactosamine	GalNAc-4 SO_4 (IdoA-2 SO_4)
Heparan sulfate	D-Glucuronic acid/L-iduronic acid and D-glucosamine	(IdoA-2 SO_4) GlcNAc-3,6 SO_4 (GlcA-2 SO_4)
Keratan sulfate	D-Galactose and N-acetylglucosamine	GlcNAc-6 SO_4 Gal-6 SO_4

Regulatory molecules for bone resorption

GAGs and/or PGs are multifunctional molecules that regulate cell behavior by fine-tuning the function of many regulatory proteins (Figure 1). For example, our data demonstrate that RANK, RANKL, OPG and PGs could form a very large complex 3:3:2:1 related to specific activities in osteoclasts [5]. The preincubation of OPG with GAG heparin inhibited, in a dose-dependent manner, OPG binding to the complex RANK–RANKL. Sulfation is essential in the OPG-blocking function of GAGs, and a decasaccharide is the minimal structure that totally inhibits OPG binding to the complex RANK–RANKL. Indeed, syndecan-1, a transmembrane PG with heparan sulfates (HS) expressed by myeloma cells, binds and sequestrates OPG [6]. Internalization and degradation of OPG after sequestration by syndecan-1 contribute to low local and systemic OPG levels in patients with multiple myeloma, which indirectly leads to bone resorption. Furthermore, syndecan-1 is involved in OPG-induced chemotaxis of human peripheral blood monocytes [7]. Overall, these data demonstrate that the PGs must be considered as essential co-factors modulating the bone remodeling in favor of bone resorption. Indeed, PGs decrease the bioavailability of OPG inducing its internalization [6,8] and enhance the RANKL half-life at the cell membrane [8].

Heparin demonstrates several kinds of biological activities by binding to various extracellular molecules and has pivotal roles in bone metabolism. Indirect proof of heparan sulfate proteoglycans' (HSPGs') involvement in osteoclast functions is given in a

TABLE 2

Structural and functional properties of some proteoglycans

Proteoglycan	GAGs (*n*)	GAG types	Core protein (kDa)	Tissue or cells	Functions
Proteoglycan extracellular matrix					
Aggrecan	~100 20–30	Chondroitin sulfate Keratan sulfate	208–221	Cartilage Blood vessel wall	Mechanical support Solute transport
Versican	1–23	Chondroitin sulfate	74–373	Fibroblasts	Mechanical support Cell migration, adhesion, proliferation
Decorin	1	Chondroitin sulfate/dermatan sulfate	8–40	Skin, bone, tendon	Cell adhesion TGF-β binding Fibrillogenesis
Biglycan	2	Chondroitin sulfate/dermatan sulfate	42	Bone, skin, dentin, tendon	Cell adhesion Fibrillogenesis
Fibromodulin	1	Keratan sulfate	43	Tendon, articular cartilage, growth plate	Cell adhesion Fibrillogenesis
Perlecan	2–15	Heparan sulfate Chondroitin sulfate	469	Basement membranes	Stability of basement membranes Provide filtration barrier
Proteoglycan cell surface					
Syndecan-1	1–3	Chondroitin sulfate Heparan sulfate	31	Epithelia, plasma cells	Mediate cell binding, cell signaling, and cytoskeletal organization
Syndecan-2	1–2	Heparan sulfate	22	Endothelia, fibroblasts	
Syndecan-4	1–2	Heparan sulfate	22	Ubiquitous on adherent cells	
Betaglycan	1–4	Chondroitin sulfate/heparan sulfate	94	Fibroblasts	TGF-β type III Receptor
Glypican-3	1–2	Heparan sulfate	65	Highly expressed in lung, liver and kidney	Modulating ligand–receptor interactions

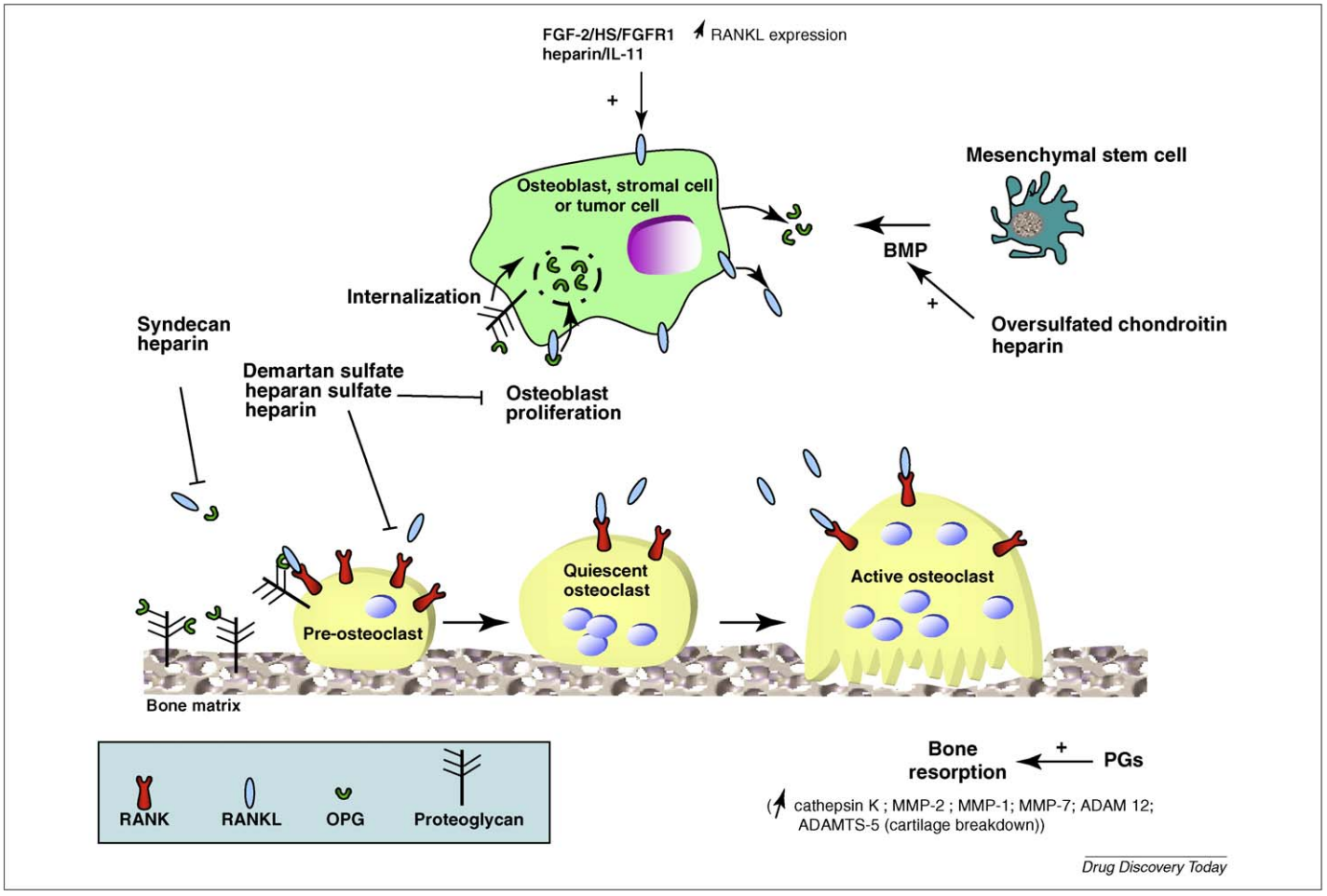

FIGURE 1

Proteoglycans (PGs) seem crucial for maintaining an appropriate number of osteoblasts and osteoclasts by modulating their proliferation and/or differentiation; however, the role of GAG and/or PGs in the biological activity of bone cells remains unclear. Thus, heparin, after binding to receptor activator of NF-κB ligand (RANKL), suppressed osteoclastogenesis and inhibited the formation of resorption pits induced by RANKL. By contrast, heparin binds to osteoprotegerin (OPG) and prevents OPG-mediated inhibition of osteoclastic bone resorption. PGs decrease the bioavailability of OPG, inducing its internalization. Like OPG, PGs interact with RANKL and abolish osteoclastogenesis. Fibroblast growth factor-2 (FGF-2) also induces, after binding to HS, the expression of RANKL and osteoclast maturation. The HS side chain and the sulfation pattern produced by the cells at specific stages of *in vitro* osteogenesis are central to the ability of the cells to mediate differentiative stimuli. PGs regulate the bone morphogenetic protein (BMP)-mediated differentiation of mesenchymal stem cells (hMSCs) into osteoblasts. *In fine*, HSPG can also participate in bone resorption regulation through the modulation of protease activity.

publication that demonstrated that heparanase, endoglycosidase expressed in osteoblastic cells, stimulates bone formation and bone mass [9]; however, the role of heparin in the biological activity of bone remains unclear. Ariyoshi *et al.* [10] showed that GAG heparin after binding to RANKL suppressed osteoclastogenesis and inhibited the formation of resorption pits induced by RANKL. By contrast, Irie *et al.* [11] showed that GAG heparin specifically binds to OPG and prevents OPG-mediated inhibition of osteoclastic bone resorption in the coculture of mouse bone marrow cells and osteoblasts. They also demonstrated that GAG heparin enhances osteoclastic bone resorption by inhibiting OPG activity. Long-term GAG heparin treatment causes cancellous bone loss in rats due, in part, to an increase in the number of osteoclasts lining the trabecular bone surface. One suggested mechanism resides in the fact that GAG heparin acts synergistically with interleukin-11 to induce STAT3 activation and *in vitro* osteoclast formation, through enhancement of IL-11's ability to induce the expression of RANKL and gp130 [12]. Besides, the GAG heparin effect GAG heparin effect is not only charge dependent but also chain-length dependent. The observation that low-molecular-weight GAG heparin has less effect on bone formation than GAG heparin is compatible with the results of clinical trials indicating that low-molecular-weight GAG heparin produces less bone loss after long-term administration [13].

GAG and/or PGS can also participate in bone resorption regulation through the modulation of protease activity. The collagenolytic activity of cathepsin K is related to a specific complex that constitutes five cathepsin K and five chondroitin sulfate molecules. This complex has a triple helical collagen-degrading activity, whereas the monomeric form of cathepsin K can degrade non-collagenous substrates [14]. GAGs predominantly expressed in bone and cartilage, such as chondroitin sulfate and keratan sulfate, enhance the collagenolytic activity of cathepsin K, whereas dermatan sulfate, HS and heparin selectively inhibit its activity. In addition, sulfated GAGs could have important roles in controlling the activation and thereby activity of matrix metalloproteinase (MMP). Via direct interaction with the promatrilysin (proMMP-7),

sulfated GAGs (such as chondroitin 4,6-sulfate) act as allosteric modulators promoting the autolytic activation of the proteinase. Once activated, GAGs can facilitate proteolysis of certain substrates by interacting with the substrate, the enzyme, or both [15]. The autolytic activation of proMMP2 and activity of MMP1 are enhanced by heparin [16], suggesting that sulfated GAGs might have wide roles in controlling MMP proteolysis. Syndecan-4, an HSPG, controls ADAMTS-5 (a disintegrin and metalloproteinase with thrombospondin motifs-5) activation through direct interaction with the protease and thereby cartilage breakdown in osteoarthritis [17]. By contrast, syndecan-2, other HSPG, acts as a suppressor for MMP-2 activation on the cell surface [18]. *In fine*, HS regulates ADAM 12 (a disintegrin and metalloproteinase domain 12) through a molecular switch mechanism. The noncovalently associated prodomain and the catalytic domain of ADAM 12 in concert form a novel molecular switch crucial for the regulation of the ADAM 12 proteolytic activity by HSPGs [19].

Modulation of cell proliferation and/or differentiation

Osteoblastic cells produce a complex ECM composed of a mixture of PGs, collagens and noncollagenous proteins (Figure 1). The interaction of PGs with matrix effector macromolecules via either their GAG chains or their protein core is crucial in regulating a variety of cellular events. Alterations in the structural composition of the GAG and/or PGs components of the ECM can have important consequences on cell proliferation and/or differentiation. Biglycan deficiency causes less bone morphogenetic protein (BMP)-4 binding and increases osteoclast differentiation and activity because osteoblasts are defective [20]. GAG heparin strongly inhibited the proliferation rates of both normal osteoblasts and transformed osteoblastic cells at concentrations $\geq$1 μg/ml. The obtained results suggest that matrix GAGs are factors that affect cell growth of both malignant and normal cells of the osteoblastic lineage in a concentration-dependent manner [21]. Recently, Haupt *et al.* [22] demonstrated the dependence of osteogenesis on specific HS chains, in particular those associated with glypican-3. Thus, when compared with non-osteogenic culture conditions, under established osteogenic culture conditions, pre-osteoblast cells increase their osteogenic gene expression profile. The osteogenic cultures decreased their chondroitin and dermatan sulfate PGs (biglycan, decorin and versican) but increased levels of the HS core protein gene expression, in particular glypican-3. Hence, the HS side chain and the sulfation pattern produced by the cells at specific stages of *in vitro* osteogenesis are central to the ability of the cells to mediate differentiative stimuli. In addition, this study clearly demonstrated an interaction between the activity of a specific HSPG and Runx2 during osteogenesis. Reduced glypican-3 abrogated the Runx2 expression and the differentiation. By contrast, the reintroduction of this HSPG into Runx2-null cells enabled osteogenesis to proceed, demonstrating the coordinate control of osteoprogenitors and their progression toward the osteogenic lineage by Runx2 and the HSPG [23]. In addition, the chlorate-induced-desulfation of GAGs expressed by MG63 cells delayed *in vitro* osteogenesis [24]. These different effects are closely related to the composition and the sulfation of GAGs.

GAG HS and GAG chondroitin sulfate directly regulate the BMP-mediated differentiation of mesenchymal stem cells into osteoblasts. BMPs, which have been shown to be heparin-binding proteins, induce osteoblast differentiation in mesenchymal stem cells [25]. Sulfated polysaccharides enhance the biological activity of both homodimers and heterodimers of BMPs by continuously serving the ligands to their signaling receptors expressed on cell membranes, such as to oversulfated chondroitin sulfate that binds to BMP-4 and enhances osteoblast differentiation [26]. GAG heparin demonstrates several kinds of biological activities by binding to various extracellular molecules and has pivotal roles in bone metabolism; however, the role of heparin in the biological activity of BMP remains unclear. Heparin suppresses BMP-2–BMPR binding and inhibits BMP-2 osteogenic activity *in vitro* [27]. Heparin stimulates BMP activity *in vitro* and enhances BMP-2-induced gene expression [28]. Simultaneous administration of BMP-2 and heparin *in vivo* dose-dependently induced larger amounts of mineralized bone tissue than BMP-2 alone. These findings clearly indicate that heparin enhances BMP-induced osteoblast differentiation not only *in vitro* but also *in vivo* [28]. This study indicates that heparin enhances BMP-induced osteoblast differentiation by protecting BMPs from degradation and inhibition by BMP antagonists. Besides, PGs sequester BMP-2 at the cell surface and mediate BMP-2 internalization. Depletion of cell surface HSPGs enhances BMP-2 morphogenetic bioactivity and the addition of exogenous heparin reduced BMP-2 signaling. These results suggest that cell surface HSPGs mediate BMP-2 internalization and modulate BMP-2 osteogenic activity [29].

The HS of the ECM play a key part during both development and wound repair in regulating the flow of growth and adhesive factors across their cell surface receptors. GAG HS bind to tumor growth factor-β1 (TGF-β1), a known inhibitor of osteoprogenitor growth, at higher affinity than a suite of other bone-related, heparin-binding growth factors [30]. This binding suggests that HS have a crucial role in regulating TGF-β availability. Overcoming such sugar-mediated inhibition might prove important for wound repair. Given the importance of HSPGs for bone metabolism, it can be anticipated that heparin, owing to its structural similarity with HS chains, somehow interferes with the biological activities of these cell surface- and ECM-associated molecules.

Taken together, these data show different levels of bone resorption regulation by GAGs, most of them leading to proresorptive effects. Therefore, modulation of PG synthesis or structure during pathological states might also interfere with bone content through dysregulation of bone resorption.

Bone tumor development

Bone is a highly hospitable environment for colonization and growth of metastatic tumors [4] (Figure 2). The interaction between tumor cells and the bone marrow microenvironment is crucial for the initiation and promotion of skeletal malignancies. These observations suggest a vicious cycle driving the formation of osteolytic bone tumors; tumor cells secrete soluble factors in bone, which stimulates osteoclastic bone resorption through indirect RANKL production by osteoblastic stromal cells. Other data, however, showed that tumor cells can themselves produce RANKL, acting directly on osteoclast differentiation and activation. Osteoclastic resorption, in turn, releases growth factors from the bone matrix, which can activate the tumor cells. In particular, TGF-β is abundant in bone matrix and released as a consequence of osteoclastic bone resorption. In addition to its rich stores of TGF-β, bone

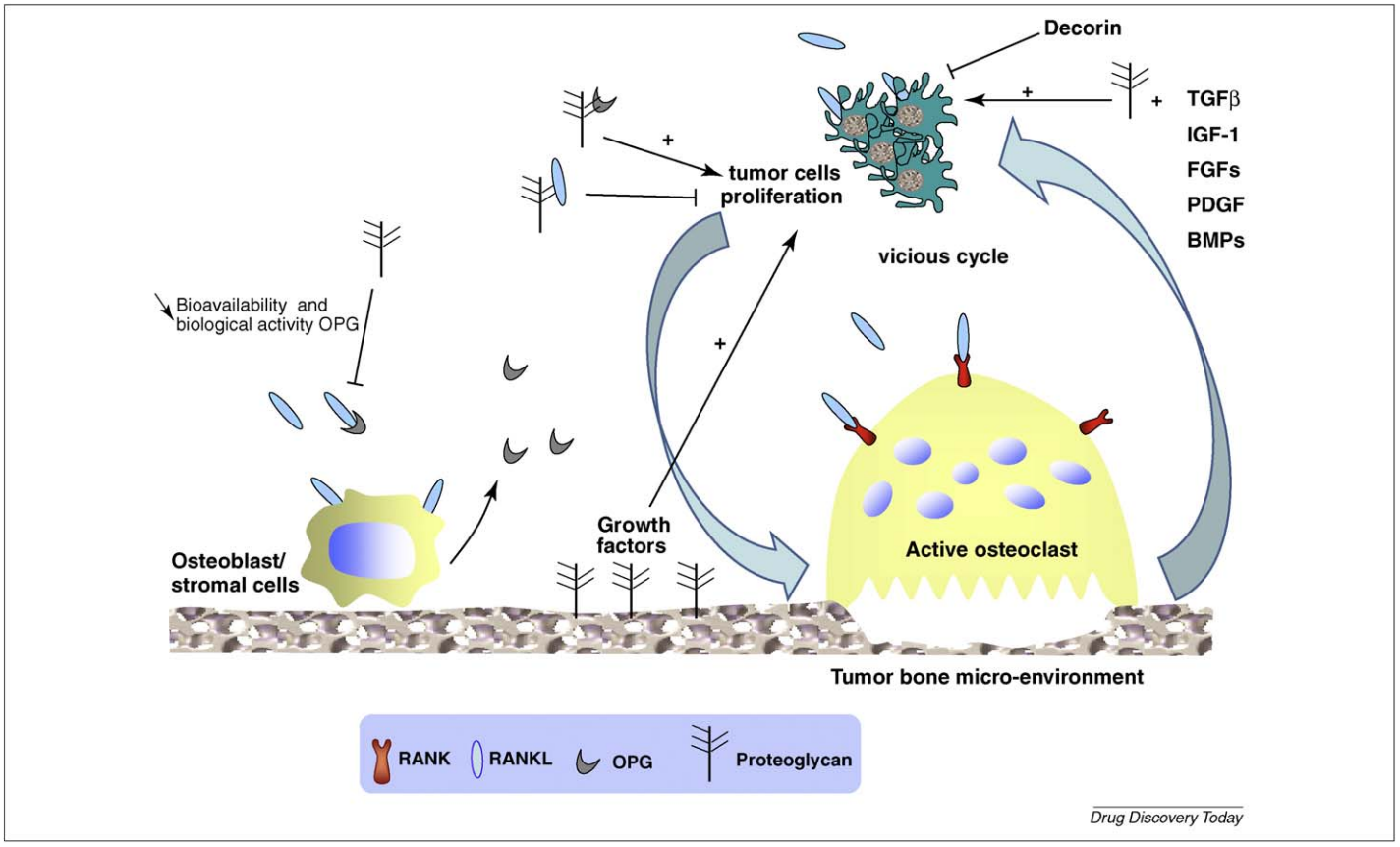

FIGURE 2

Tumor cells can release soluble mediators such as cytokines, growth factors that act on osteoblastic stromal cells and osteoclast precursor cells. Active osteoclasts then release growth factors, cytokines or bone matrix components stored in the bone matrix that, in turn, activate tumor cell proliferation. The vicious cycle thus induces both tumor cell proliferation and bone resorption. Heparan sulfate proteoglycans (HSPGs) have important roles in regulating disease processes, including cancer. Although early studies provided evidence that HS acts to suppress the malignant phenotype, evidence is growing that HS can also promote tumor growth and metastasis by sequestering chemokines or growth factors within close proximity to migrating tumor cells. Cell surface HSPGs serve as co-receptors for several growth factor receptors and contribute to cancer cell proliferation. By contrast, decorin affects the biology of various types of cancer by downregulating the activity of several receptors involved in cell growth and survival. These overall results suggest different levels of bone resorption regulation by GAG and/or PGs: directly, through the regulation of OPG bioavailability and biological activity, and indirectly, on RANKL activity in bone tumor environment.

contains other growth regulatory factors that can act as tumor growth factors, including BMP, heparin-binding fibroblast growth factors and insulin-like growth factor I. The consequences of this vicious cycle are an increased tumor cell proliferation paralleled with an imbalance of bone formation/bone resorption ratio in favor of bone destruction.

GAGs and PGs are major constituents of the ECM, and cell surface PGs mediate cell–matrix interactions. It has become increasingly clear that HSPGs have important roles in regulating disease processes, including cancer. Although early studies provided evidence that HSPGs act to suppress the malignant phenotype [31], evidence is growing that HSPGs act can also promote tumor growth and metastasis by sequestering chemokines or growth factors within close proximity to migrating tumor cells [32]. Cell surface HSPGs serve as co-receptors for several growth factors tyrosine kinase receptors and contribute to cancer cell proliferation. Syndecans can act as co-receptors to modulate integrin-mediated cell–matrix adhesion as, for example, syndecan-4 co-localizes in focal adhesions with integrin β1 subunits [33]. PGs can accumulate within the ECM or diffuse into the circulation and reach high levels in some cancer patients. The HSPGs trap and concentrate growth and chemotactic factors within the tumor microenvironment. This enhances angiogenesis and promotes tumor growth and osteoclastogenesis, resulting in bone destruction. The evidence indicates that syndecan-1 is a key mediator of cross-talk between tumor cells and bone, thereby sparking events that ignite the 'vicious cycle' of lytic bone disease, in which tumor cells undergo rampant growth with associated increased osteoclastogenesis [34]. By contrast, decorin, a member of small leucine-rich repeat PGs, affects the biology of various types of cancer by downregulating the activity of several receptors involved in cell growth and survival [35]. As an exception, decorin is not capable, in MG-63 human osteosarcoma cells, of reducing tumor cell growth but favors cell migration by blocking TGF-β2 effects [36]. Decorin-expressing tumor xenografts grew at markedly lower rates and showed a statistically significant suppression of neovascularization compared to their wild-type counterparts, certainly caused by the reduced rate of VEGF production in decorin-expressing cells [37]. These results provide a mechanism of action for decorin and indicate that decorin could adversely affect *in vivo* tumor growth by suppressing the endogenous tumor cell production of a powerful angiogenic stimulus.

We have recently shown *in vivo* that GAG and/or PGs highly expressed in osteosarcoma microenvironment could regulate OPG

bioactivity [38]. In this model, we suggest that GAG and/or PGs could have a role as inhibitors of OPG activity through its sequestration in tumor bone matrix. These overall results suggest different levels of bone resorption regulation by GAG and/or PGs: directly, through the regulation of OPG bioavailability and biological activity, and indirectly, on RANKL activity in bone tumor environment [3] because surface plasmon resonance studies revealed that GAGs were able to disturb OPG–RANKL interaction.

Therapeutic approaches

Because the anticoagulant activity of heparin is dominating, its therapeutic use for other medical indications is limited because of an associated risk of bleeding. Further disadvantages of heparin are its animal origin, the shortage of resources, and its complex and variable composition. However, a prognostic value for the clinical outcome of cancer has recently been assigned to changes in expression of several GAGs and PGs [39]. These findings have raised considerable interest in the generation of GAG and/or PGs-based diagnostic tools. Knowledge of 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 GAGs with defined structures. Thus, in therapeutics, targeting of GAGs and PGs and the use of GAGs, PGs and their mimetics are highly promising.

Chondroitin sulfate/dermatan sulfate have intriguing biological activities, which, in turn, should help in the development of GAG mimetic-based therapeutics. They function as regulators of functional proteins such as growth factors, cytokines, chemokines, adhesion molecules and lipoproteins through interactions with the ligands of these proteins via specific saccharide domains [40]. Structural alterations have often been implicated in pathological conditions, such as cancer and atherosclerosis. Recent microsequencing of oligosaccharides that bind growth factors, such as pleiotrophin, and various monoclonal antibodies against chondroitin sulfate/dermatan sulfate have revealed a considerable number of unique oligosaccharide sequences [41]. The detection and identification of such oligosaccharide sequences would be an important contribution to cancer therapy and open up the potential to produce GAGs or GAG mimetics, with greater specificity and fewer side-effects than crude heparin or chondroitin sulfate preparations.

GAGs frequently act as co-factors in ligand binding to receptors and, therefore, if added exogenously, they can act as agonists, promoting signaling, but can also be antagonists, by preventing ligand–receptor engagement. Thus, angiogenesis promoted by the VEGF has been reported to require endogenous expression of fibroblast growth factor (FGF) by endothelial cells, and it is, therefore, blocked by neutralizing antibodies against FGF [42]. Consequently, inhibition of FGF mitogenic activity seems a crucial target for the development of antiangiogenic cancer treatments. Prevention of the formation of the HS:FGF:FGFReceptor ternary complex via blocking the interaction of HS with the FGF might, therefore, form the basis of antiangiogenic therapies. Several structurally simple heparin mimetics bind to the FGFs in the HS binding site and inhibit FGF mitogenic activity [43]. Thus, disruption of the interaction of FGFs with heparin and HS seems an obvious target for antiangiogenesis.

Sulfated oligosaccharides, which are structural mimics of HS, might also inhibit cleavage of HS by heparanase. Heparanase is preferentially expressed in human tumors and its overexpression in tumor cells confers an invasive phenotype in experimental animals [44]. The enzyme also releases HS-bound angiogenic growth factors from the ECM and thereby induces an angiogenic response *in vivo*. Heparanase upregulation correlates with increased tumor vascularity and poor postoperative survival of cancer patients. Heparanase stimulates expression of MMP-9 and u-Plasminogen activator/uPAReceptor, which catalyze shedding of syndecan-1 from the cell surface, thereby regulating both the level and the location of syndecan-1 within the myeloma microenvironment [45]. This suggests that clinical inhibitors of heparanase could be beneficial for patients with cancer.

The phosphosulfomannan (PI-88) is a potent inhibitor of the enzyme heparanase and acts as a HS decoy or inhibitor of heparanase [46]. PI-88 is a mixture of highly sulfated, monophosphorylated mannose oligosaccharides ranging in size from di- to hexasaccharide derived from the yeast *Pichia* (*Hanensula*) *holstii* NRRL Y-2448 [47]. The major components are penta- (~60%) and tetrasaccharides (~30%). PI-88 exerts antiangiogenic effects by inhibiting the interactions of proangiogenic growth factors and their receptors with HS [48]. PI-88 had notable effects at distinct stages of tumorigenesis, producing a reduction in the number of early progenitor lesions and an impairment of tumor growth at later stages. These responses were associated with decreased cell proliferation, increased apoptosis, impaired angiogenesis and a substantive reduction in the number of invasive carcinomas. PI-88 has shown activity against melanoma in phase I studies [49]. This was an open-label, multicenter, phase II study of PI-88 in patients with advanced melanoma. Although the current study did not meet the primary end-point of progression-free survival of $\geq$20%, there is some evidence of activity and further investigation is warranted [50]. The mechanism of PI-88 inhibition was not merely confined to the antiheparanase activity of this compound. Thus, studies by surface plasmon resonance revealed that PI-88 potently inhibited the interaction of FGF-2 with HS and show the capacity of this sulfated oligosaccharide to directly bind FGF-2, block extracellular signal-regulated kinase (ERK)-1/2 activity and proliferation *in vitro* [51].

Recently, it was shown that PI-88 reduced growth plate expansion and increased bone marrow cavity proportion of cultured metatarsals. These results suggest that heparinase activity aided the transition from chondrogenic to osteogenic processes in growth of long bones. PI-88 also decreased cell migration in proliferative and terminally differentiated chondrocytes. The inhibition of heparinase activity by PI-88 *in vitro* and *ex vivo* leads to premature termination of chondrogenic growth and differentiation and favors premature osteogenic differentiation [52].

A broad series of polysaccharides has emerged as an important class of bioactive products [53] that occur naturally in a great variety of animals, plants and microorganisms. Marine microorganisms offer a rich source of polysaccharides, such as carrageenans from red algae or fucoidans from brown algae. Interest in mass culture of microorganisms from the marine environment has increased considerably, representing an innovative approach to the biotechnological use of under-exploited resources [54]. Such natural polysaccharides might be suitable as ingredients

for cosmetics or food supplements but not for the development of medical products; they would hardly meet the current high requirements on pharmaceutical quality owing to their complex structure composition and biovariability. By contrast, chemically defined synthetic or semisynthetic sulfated polysaccharides might be promising for the development of new drug substances. For example, PS3, a semisynthetic β-1,3-glucan sulfate derived from the red algae *Delesseria sanguinea*, has both anti-inflammatory [55] and antimetastatic effects on animal models [56] without the disadvantages of heparins. Another, the marine-derived oligosaccharide oligomannurarate sulfate (JG3), acts as a heparanase inhibitor. JG3 significantly inhibited tumor angiogenesis and metastasis, both *in vitro* and *in vivo*, by combating heparanase activity via binding to the heparanase molecule. The JG3–heparanase interaction was competitively inhibited by low-molecular-weight heparin but not by other GAGs. JG3 seems to inhibit both major heparanase activities by acting simultaneously as a substrate mimetic and as a competitive inhibitor of heparan sulfate [57]. Besides, JG3 abolished heparanase-driven formation of focal adhesions and cell spreading [58]. The oligosaccharide causes a statistically significant dephosphorylation of focal adhesion kinase and subsequent inactivation of Erk, thereby decreasing the adhesion and invasion of cancer cells via ERK-1/2 signaling pathway activation [59]. These findings help form an alternative view to understand the mechanisms underlying the inhibitory effects of JG3 on cell motility. JG3 and PS3 represent promising candidates for further development as anti-inflammatory or anti-metastatic drugs [58,60].

Progress in the development of GAG and/or PGs mimetics that act only on specific steps of tumor progression will enable more selective therapy. These molecules represent new tools for understanding some of the different interactions described above and open a whole new area of development in the treatment of inflammation and bone diseases. These approaches combined with conventional chemotherapy have already shown synergistic effects in cancer treatments. In the near future, additional studies are required to validate these therapies in preclinical models and to determine their clinical safety and efficacy.

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