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Experimental Hematology

Experimental Hematology 2016;44:1002–1012

REVIEW

Mechanisms of heparanase inhibitors in cancer therapy

Benjamin Heyman[a] and Yiping Yang[a,b]

[a]Division of Hematologic Malignancies and Cellular Therapy, Department of Medicine, Duke University, Durham, North Carolina, USA;
[b]Department of Immunology, Duke University, Durham, North Carolina, USA

(Received 15 June 2016; revised 9 August 2016; accepted 19 August 2016)

Heparanase is an endo-β-D-glucuronidase capable of cleaving heparan sulfate side chains contributing to breakdown of the extracellular matrix. Increased expression of heparanase has been observed in numerous malignancies and is associated with a poor prognosis. It has generated significant interest as a potential antineoplastic target because of the multiple roles it plays in tumor growth and metastasis. The protumorigenic effects of heparanase are enhanced by the release of heparan sulfate side chains, with subsequent increase in bioactive fragments and cytokine levels that promote tumor invasion, angiogenesis, and metastasis. Preclinical experiments have found heparanase inhibitors to substantially reduce tumor growth and metastasis, leading to clinical trials with heparan sulfate mimetics. In this review, we examine the role of heparanase in tumor biology and its interaction with heparan surface proteoglycans, specifically syndecan-1, as well as the mechanism of action for heparanase inhibitors developed as antineoplastic therapeutics.

The extracellular matrix (ECM) is composed of different proteins that maintain cellular organization and architecture. It was initially felt to be inactive, but later appreciated as a dynamic entity, where significant cell signaling interactions occur [1]. The ECM contains heparan sulfate proteoglycans (HSPGs), collagen, fibronectin, laminin, and growth factors [1]. HSPGs are ubiquitous macromolecules that are integral parts of normal tissue architecture. They possess various functions, including cell attachment/adhesion, components of structural integrity, and reservoirs for growth factors; and act as cofactors in signaling pathways [2,3]. HSPGs are composed of a core protein attached to one of several negatively charged polysaccharide chains of heparan sulfate glycosaminoglycans (GAGs). Heparan sulfate (HS) is composed of repeating units of glucosamine and glucuronic/iduronic acid residues [4].

Heparanase is an endo-β-D-glucuronidase that cleaves HS side chains. This results in structural changes and the release of bioactive HS fragments from the ECM [5]. Over the past two decades, much work has been dedicated to examining the role of heparanase in cancer biology. Various methods of analysis have revealed that heparanase expression is augmented in numerous cancers, including hematologic malignancies, carcinomas, and sarcomas [6–15]. Furthermore, elevated heparanase levels are associated with reduced postoperative survival, increased angiogenesis, and metastasis [8,12,13,16]. All of these factors have sparked the development of heparanase inhibitors as novel anticancer agents. In this article, we review the function of heparanase in cancer biology and focus on the development of heparanase inhibitors, their specific mechanism of action, and relevant clinical findings to date.

Heparanase and heparan sulfate/syndecan-1 axis

Mammalian cells express a single functional heparanase enzyme, heparanase-1 [17]. Heparanase-2, a heparanase homolog, was cloned but is incapable of HS-degrading activity [18,19]. It may, however, regulate heparanase-1 activity [20]. The heparanase gene is located on chromosome 4q21.3 and is highly conserved throughout different species [21]. It is first expressed as pre-proheparanase, with the N-terminal signal removed on translocation to the endoplasmic reticulum, generating a 65-kDa proheparanase; it is then moved to the Golgi apparatus, where it is

Offprint requests to: Dr. Yiping Yang, Department of Medicine, Duke University Medical Center, Box 103005, Durham, NC 27710; E-mail: yang0029@mc.duke.edu

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http://dx.doi.org/10.1016/j.exphem.2016.08.006

encapsulated and secreted. Once secreted, it interacts with extracellular components before being internalized and mobilized to the late endosome/lysosome where it undergoes posttranslational proteolysis and alternative splicing to become active heparanase [22–25]. The active form of heparanase consists of a heterodimer composed of 8- and 50-kDa subunits that are noncovalently liked. The heparanase structure contains a TIM barrel fold, which incorporates the enzyme's active site, and a distinct C-terminus domain that has noncatalytic properties and is involved in heparanase's nonenzymatic signaling and secretory function [26–28]. Recently, the human heparanase enzyme structure was solved, confirming the TIM barrel fold structure [29].

Heparanase expression is under tight regulation. In noncancerous cells, the heparanase promoter is constitutively inhibited secondary to promoter methylation and activity of wild-type p53, which suppresses transcription of the heparanase gene by directly binding to its promoter [30]. Furthermore, additional regulation occurs during posttranslational processing. Cathepsin L is necessary for posttranslational activation of heparanase, and inhibitors of cathepsin L impede the formation of active heparanase [31]. In nonpathologic states, heparanase expression is restricted primarily to platelets, activated white blood cells, and the placenta, with little or no expression in connective tissue or normal epithelium [5]. Moreover, it is most active under acidic conditions (pH 5–6), during inflammation or within the tumor microenvironment [16].

The syndecans (SDCs) are a family of four HSPGs that are either membrane bound or soluble. They have diverse functions including cell differentiation, cell adhesion, cytoskeletal organization, cell migration/invasion, and angiogenesis [32–35]. Syndecan-1 (SDC-1) has been the most extensively studied and is found principally on epithelial cell surfaces. However, it is also present during different stages of lymphoid development, specifically on pre-B cells and plasma cells [36,37]. Loss of both syndecan-1 and E-cadherin from the cell surface is considered an integral step in neoplastic epithelial–mesenchymal cell transition [38].

The heparanase/SDC-1 axis is a key regulator of cell signaling within tumor cells and the microenvironment, especially in multiple myeloma [39]. Syndecan-1 is made of three domains: (1) an extracellular domain composed mostly of heparan sulfate GAGs; (2) a transmembrane domain; and (3) a highly conserved cytoplasmic domain [40]. Syndecan-1 can be shed and mobilized via proteolytic cleavage of the extracellular domain near the plasma membrane. This is performed primarily by shedases, frequently matrix metalloproteinases (MMPs) [41]. Shed syndecan-1 contains bound HS chains within the ectodomain (which typically contain bound growth factor) and, thus, can become a paracrine signaler by transferring signaling proteins from one cell to another [41]. In the case of malignancy, this is often from a cancer cell to a stromal cell [42,43]. Syndecan-1 shedding is regulated by various extracellular mechanisms including heparanase, growth factors (fibroblast growth factor-2 [FGF-2]), and chemokines [44–46].

Heparanase increases syndecan-1 shedding, both in human myeloma and in breast cancer cell lines, by augmenting expression of MMP-9 through upregulation of ERK phosphorylation [47]. Heparanase also reduces the length of HS chains attached to syndecan-1, enhancing the rate at which shedases cleave the core protein [47]. Syndecan-1 is also shed constitutively, which is accelerated in tumors, typically in response to growth factors, chemokines, or other agonists [48]. Recently, it was found that chemotherapy stimulates syndecan-1 shedding in colorectal cancer, pancreatic cancer, and human myeloma cell lines, increasing the risk for relapse and chemotherapy resistance [49,50].

The heparanase/syndecan-1 axis regulates growth factor release, thus modulating cellular proliferation [51]. Both hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) are regulated by the heparanase/syndecan-1 axis. HGF is a cytokine that enhances growth, motility, and angiogenesis of tumor cells [52]. Heparanase has been found to increase expression of HGF in myeloma cell lines. Shed syndecan-1 binds to secreted HGF, facilitating a paracrine and autocrine signaling cascade via cell surface receptor c-Met [52]. Similarly, heparanase enhances VEGF secretion from tumor cells. Secreted VEGF subsequently binds shed sydecan-1 in the ECM, stimulating angiogenesis and endothelial invasion via the Erk pathway [43]. In breast cancer, shed syndecan-1 promotes angiogenesis and growth via activation of FGF2 [42]. In multiple myeloma, shed syndecan-1 in the bone marrow ECM enhances growth, angiogenesis, and metastasis of myeloma cells within the bone. Cell membrane syndecan-1 promotes myeloma cell adhesion and inhibits invasion. Conversely, heparanase facilitates invasion of myeloma by increasing the expression and shedding of syndecan-1 [43,47,53].

Heparanase and syndecan-1 can also be transported to the nucleus to regulate gene expression. Shed syndecan-1 and the full syndecan-1 protein have been identified in the nucleus [51]. Similarly, HS has also been identified in the nucleus, both as free chains and bound to syndecan-1. Syndecan-1 transports HS to the nucleus, as it does for FGF2. In general, nuclear HS and syndecan-1 are antiproliferative and decrease gene transcription. Specifically, highly sulfated nuclear HS chains are mostly inhibitory [51,54]. This is in contrast to extracellular shed syndecan-1, which promotes cell migration, angiogenesis, invasion, and proliferation [51]. Once in the nucleus, HS can regulate gene expression by decreasing histone acetylation and inhibiting transcription factors [55]. Both syndecan-1 and HS can inhibit histone acetyl transferase enzyme (HAT), reducing gene expression and tumor growth [56,57]. Conversely, heparanase augments gene expression in the nucleus and promotes growth [58]. In T-lymphocytes, heparanase binds to euchromatin, altering gene transcription [58]. Heparanase increases DNA topoisomerase I activity in metastatic