Involvement of Heparanase in Tumor Progression and Normal Differentiation

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Mammalian Heparanase

Despite of earlier reports on the existence of several distinct mammalian HS degrading endoglycosidases (heparanases), the cloning of the same gene by several groups suggests that mammalian cells express primarily a single dominant functional heparanase enzyme. The mature active 50 kDa enzyme, isolated from cells and tissues, has its N-terminus 157 amino acids downstream from the initiation codon, suggesting post-translational processing and activation of the heparanase polypeptide [1, 2]. We have recently demonstrated that apart of its heparan sulfate-degrading activity, the heparanase molecule also mediates cell adhesion and signal transduction, independent of its enzymatic activity [3].

Involvement in Cancer Metastasis and Angiogenesis

Despite of earlier reports on the existence of several distinct mammalian HS degrading endoglycosidases (heparanases), the cloning of the same gene (*Hpa-1*) by several groups suggests that mammalian cells express primarily a single dominant functional heparanase enzyme. Expression of the human heparanase mRNA in normal

tissues is restricted primarily to the placenta, cells of the immune system and keratinocytes. Preferential expression of the *hpa* mRNA and protein was clearly demonstrated in tissue specimens derived from adenocarcinoma of the ovary, metastatic melanoma, oral squamous cell carcinoma, hepatocellular carcinoma and carcinomas of the colon, breast, prostate, bladder, intestine and pancreas. Patients exhibiting high levels of the *hpa* mRNA had a significantly shorter postoperative survival time than patients whose tumors contained relatively low levels of heparanase. There is also a significant correlation between *hpa* mRNA expression levels and tumor vascularity in cancer patients [4].

The ability of HSPGs to interact with various ECM macromolecules and with different attachment sites on plasma membranes suggests a key role for this proteoglycan in the self-assembly and barrier function of ECM components, as well as in cell adhesion and locomotion. Expression of heparanase correlates with the metastatic potential of human tumor cells [1, 2]. Moreover, non-anticoagulant and chemically modified species of heparin and other polysaccharides that inhibit heparanase activity (see below), also inhibit experimental metastasis. Apart of direct involvement in basement membrane (BM) invasion by endothelial cells, heparanase elicits an indirect angiogenic response by releasing HS-bound angiogenic growth factors (i.e., bFGF, VEGF) from the tumor

microenvironment, and by generating HS fragments which potentiate bFGF receptor binding, dimerization and signaling [5]. Heparanase may also be involved in neovascularization through mobilization of endothelial progenitor cells from the bone marrow into target organs. Altogether, the enzyme fulfills important roles in tumor progression.

Normal Development and Tissue Remodeling

While most studies emphasize the involvement of heparanase in pathophysiology, little is known about the enzyme's contribution to normal cell and tissue function. Our studies with heparanase over-expressing mice indicate that among the functions of heparanase in the adult are HS turnover, immune surveillance, tissue repair, regeneration and vascularization, cell mobilization and engraftment, and other processes involving tissue morphogenesis (i.e., mammary glands) and remodeling (i.e., bone formation, hair follicle growth).

Modulation of Heparanase-Inhibiting Activity of Heparin Through Selective Desulfation, Graded N-Acetylation and Glycol Splitting

Mimics of HS (heparin and heparin derivatives, and other sulfated oligo- and polysaccharides) have been investigated as heparanase inhibitors and some of them have shown antimetastatic activity in animal models [1, 2, 6]. Both the type of the polysaccharide backbone and degree of sulfation seem to affect the heparanase-inhibiting activity of sulfated polysaccharides. However, different heparin preparations display significantly different anti-heparanase activity, indicating that this activity is also dependent on more subtle structural features. The structure of heparin is largely accounted for by sequences of trisulfated disaccharide (TD) units made up of α -linked L-iduronic acid 2-sulfate and D-glucosamine N,6-disulfate, with minor sequences constituted by D-glucuronic and N-acetylated glucosamine residues. Heparin fractions and modified species of heparin differing in degrees of N-acetylation, 2-O-sulfation, and glycol splitting of nonsulfated uronic acid residues were tested for their heparanase-inhibiting activity. Within the unmodified heparin series, the strongest heparanase inhibitors were fractions with the highest content of TD sequences. N-desulfation/N-acetylation involved a progressive decrease in inhibitory activity. However, glycol splitting of nonsulfated uronic acid residues significantly increased this activity both for

otherwise nonmodified heparins and for N-acetylated heparins, irrespective of the degree of N-acetylation. Partially 2-O-desulfated and glycol split derivatives of heparin inhibited both heparanase activity and experimental metastasis (B16/BL6 melanoma) significantly more than the original heparins. Since glycol splitting also involves inactivation of the active site for antithrombin [7], these derivatives have very low anticoagulant activity. Some of them are also potent inhibitors of FGF-2 and are antiangiogenic in experimental animal models [8], thus qualifying as potential antimetastatic drugs.

Expression of the Heparanase Gene in Leukemic Promyelocytes is Regulated by Vitamin D and All-Trans-Retinoic-Acid (ATRA)

Heparanase activity was found in several types of terminally differentiated hematopoietic cells, including neutrophils, monocytes, megakaryocytes and activated lymphocytes [9]. The enzyme mediates cell extravasation during inflammatory and immune responses and hence may be important for the normal function of mature myeloid cells. We have previously characterized heparanase expression in human leukemias [10]. Heparanase expression was detected in acute myeloid leukemias, but was often absent from chronic myeloid leukemia cells and several types of lymphoid leukemias [10]. Heparanase expression was found in mature monocytes and granulocytes, but was not detected in immature hematopoietic progenitors (CD34⁺) [10]. It appears that heparanase expression may be associated with normal myeloid differentiation, reaching high levels in mature myeloid cells. In order to examine the regulatory mechanisms of heparanase expression, we utilized HL-60 and NB-4 promyelocytic cell lines. These cell lines express very low levels of heparanase RNA. The cells were treated with two differentiating agents: All-Trans-Retinoic-Acid (ATRA) and vitamin D. Expression of heparanase RNA and enzymatic activity was detected after 9 h of treatment with vitamin D and 24–48 h treatment with ATRA. We then examined whether ATRA may affect heparanase expression in the clinical setting as well. A blood sample taken from a patient with acute promyelocytic leukemia (APL), revealed a low basal level of heparanase RNA expression. After the patient was treated with ATRA, as part of a standard APL regimen, there was a marked elevation in the expression of heparanase RNA. These findings suggest that retinoic acid and vitamin D regulate the expression of heparanase RNA and activity, in correlation with myeloid differentiation.

Leukomogenic Factors Down Regulate Heparanase Expression by Histone Deacetylation

Chromatin modification by histone acetyltransferase (HAT) or histone deacetylase (HDAC) represents a key mechanism of transcriptional regulation, specifically in hematopoietic cells. Disruption of the normal control of chromatin remodeling gives rise to dis-regulated transcription, and in some cases to leukemic transformation. We found that PML-RAR α and PLZF-RAR α fusion gene products associated with acute promyelocytic leukemia abrogated myeloid differentiation and heparanase expression. AML-Eto, a translocation product associated with AML FAB M2, also down-regulated heparanase gene expression. The common mechanism that underlines the aberrant differentiation associated with these three fusion gene products involves the recruitment of histone

deacetylase complexes to specific locations within the DNA. Hence, dissociation of these molecular complexes by retinoic acid, or inhibition of histone deacetylase by trichostatin A (TSA) are expected to restore gene expression. We found that retinoic acid that dissociates PML-RAR α from the DNA, and which is used to treat APL patients, restores heparanase expression to normal levels. On the other hand, PLZF-RAR α mediated down-regulation of heparanase expression is insensitive to retinoic acid treatment, unless applied in combination with the histone deacetylase inhibitor TSA. TSA also reversed the down regulation of heparanase expression induced by the AML-Eto fusion gene product in M2 type AML. These results indicate that in certain types of leukemia, heparanase expression is suppressed by fusion gene products that inhibit histone acetylation and is controlled to a large extent by the chromatin structure, determined by histone acetylation.

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