The impact of heparanase on cancer progression

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Background

Heparanase is an endo-β-D-glucuronidase involved in cleavage of heparan sulfate (HS) and hence in degradation and remodeling of the extracellular matrix (ECM).[1,2] Heparanase activity has been traditionally correlated with the metastatic potential of tumor-derived cell types and with cell invasion associated with autoimmunity, inflammation, and angiogenesis. The heparanase gene and protein are overexpressed in a variety of human primary tumors, including those of the bladder, breast, prostate, colon, gastrointestinal system, oral cavity, esophagous, pancreas, and ovary, as well as in multiple myeloma and acute myeloid leukemia.[1,2] Heparanase upregulation is often correlated with increased tumor vascularity and poor postoperative survival of cancer patients. These observations, the anti-cancerous effect of heparanase gene silencing[3] and of heparanase-inhibiting molecules[4] and the unexpected identification of a single predominant functional heparanase, suggest that the enzyme is a promising target for anticancer drug development.

Processing and activation

Heparanase is synthesized as a 65 kDa non-active precursor that subsequently undergoes proteolytic cleavage, yielding 8 kDa and 50 kDa protein subunits that heterodimerize to form an active enzyme.[5] Processing is brought about by cathepsin L-like proteases and involves a stepwise degradation and complete removal of a ~6 kDa linker peptide, residing in between the two subunits.[5] It appears that pre-pro-heparanase is first targeted to the ER lumen via its own signal peptide. The resulting 65 kDa pro-heparanase is then shuttled to the Golgi apparatus, and is subsequently secreted. Secreted heparanase rapidly interacts with cell membrane HSPG such as syndecan-family members, followed by a rapid endocytosis of the heparanase-HSPG complex and its processing in late endosomes.[6] The lysosomal compartment may serve as a site for heparanase confinement within the cells, limiting its secretion and uncontrolled extracellular activities associated with tumor metastasis and angiogenesis. Identification of amino acids and regions essential for proteolytic processing and activation of the enzyme, as well as for substrate recognition and interaction between the 8 and 50 kDa subunits, yielded a 3D model of the heparanase enzyme, currently used to design inhibitory molecules.[5]

Inhibitory strategies

The heparanase inhibiting effect of heparin derivatives differing in degrees of 2-O- and 6-O-sulfation, N-acetylation, and glycol-splitting of nonsulfated uronic acid residues, was determined. N-desulfation/N-acetylation involved a marked decrease in the inhibitory activity for degrees of N-acetylation higher than 50%, suggesting that at least one NSO_3 group per a disaccharide unit is involved in interaction with the enzyme.[4] On the other hand, glycol-splitting of preexisting or of both preexisting and chemically generated nonsulfated uronic acids dramatically increased the heparanase-inhibiting activity, irrespective of the degree of N-acetylation.[4] Indeed, N-acetylated heparins in their glycol-split forms inhibited heparanase as effectively as the corresponding N-sulfated derivatives. Glycol-split N-acetyl heparins did not release FGF-2 from ECM and failed to stimulate its mitogenic activity.[4] The combination of high inhibition of heparanase and low release/potentiation of ECM-bound growth factors points to N-acetylated, glycol-split heparins as potential anti-angiogenic and anti-metastatic agents, more effective and specific than their counterparts with unmodified backbones.[4] We have also generated heparanase-inhibiting antibodies and peptides,[7] as well as anti-heparanase ribozyme and siRNA.[3] The latter agents inhibited tumor metastasis and angiogenesis in mouse melanoma and lymphoma models.[3]

Procoagulant activity. Tissue factor (TF)

expression correlated with heparanase activity in blasts collected from 22 acute leukemia patients. Moreover, over expression of heparanase in tumor cell lines, or addition of recombinant heparanase to endothelial or tumor-derived cells resulted in increased TF expression. Heparanase also released tissue factor pathway inhibitor (TFPI) from the surface of human umbilical vein endothelial cells (HUVEC), altogether resulting in an enhanced procoagulant effect.

Non-enzymatic functions. Heparanase exhibits also non-enzymatic activities, independent of its ECM degrading activity. For example, heparanase enhances Akt signaling and stimulates phosphatidylinositol 3-kinase-dependent endothelial cell migration and invasion.[8] The enzyme may thus activate endothelial cells and elicits angiogenic and survival responses. Heparanase was identified within the cell nucleus, capable of degrading nuclear HS, and possibly affecting nuclear functions (i.e., gene transcription) associated with cell differentiation.[9] Studies with heparanase overexpressing transgenic mice[10] revealed the involvement of heparanase in embryonic implantation, mammary and salivary gland morphogenesis, wound angiogenesis, hair follicle growth, bone formation, and resistance to amyloid[11] and prion diseases. The enzyme may thus function in normal cell mobilization, tissue vascularization and remodeling.

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