Published in final edited form as:
Exp Eye Res. 2010 October ; 91(4): 476–477. doi:10.1016/j.exer.2010.05.004.

Focus on Molecules: Heparanase

Yinghui Zhang[a], **Denise S. Ryan**[b], **Kraig S. Bower**[b], **Neta Ilan**[c], **Israel Vlodavsky**[c], and **Gordon W. Laurie**[a,*]

[a]Department of Cell Biology, University of Virginia, P.O. Box 800732 UVa Health System, Charlottesville VA, 22908-0732 USA

[b]Center for Refractive Surgery, Walter Reed Army Medical Center, Washington DC, 20307-5001 USA

[c]Cancer and Vascular Biology Research Center, Rappaport Faculty of Medicine,Technion, Haifa 31096, Israel

Keywords

dry eye; cornea; tears; syndecan; HPSE; cancer; inflammation; ocular surface

1. Structure

Heparanase (NP_001092010) is a heparin-binding endo-β-D-glucuronidase expressed by normal lacrimal gland (GEO Profiles: GDS1361), corneal epithelia (Berk et al, 2004), retinal pigment epithelium/choroid, lens (NEIBank) and some non-ocular tissues. Heparanase is over-expressed in essentially all human tumors examined. Western blotting suggests that heparanase is a constituent of normal tears (Ma, Laurie, unpublished). 119 +/− 37 pg/ml of active heparanase has been detected in normal saliva. Heparanase belongs to the glycoside hydrolase family 79. The heparanase gene 'HPSE' comprises fourteen exons over 39.8 kb of human chromosome 4, and forty-three orthologues have been documented (Ensembl release 57). HPSE is well-conserved from bony fish to human. Secreted heparanase in humans is 508 amino acids long, with a theoretical molecular weight and pI of 57.7 kDa and 9.2, respectively. Migration in SDS PAGE is approximately 65 kDa, in keeping with N-linked high mannose glycosylation at as many as six predicted sites (NetNGlyc 1.0). No O-glycosylation is predicted above threshold (NetOGlyc 3.1). Three splice variants have been recently reported (Barash et al, 2010) which vary in amino acid length, predicted molecular weight and charge as follows: 1) 'T5': 15.6 kDa and pI 6.9; 2) 'T4': 28.3 kDa and pI 8.8; and 3) 'Skip 10': 39.2 kDa and pI 8.4. Several other splice variants are predicted by Aceview. Heparanase activation is a two-step process of secretion and endocytic uptake. Secretion requires a disulfide bond between Cys437 and Cys542. Endocytocytic uptake subjects heparanase to cathepsin L excision of a 6 kDa linker segment between Ser110 and Gln157, thereby releasing N- (8 kDa) and C- (50 kDa) terminal subunits that subsequently heterodimerize into a TIM barrel fold (Fig. 1, residues 36-417) characteristic of other glycosidases. The fold, together with a 130 amino acid C-terminal domain, is responsible for binding and cleaving heparan sulfate (Fux et al, 2009).

*Corresponding author: glaurie@virginia.edu (G.W. Laurie).

Similar to other glycosyl hydrolases, heparanase has a common catalytic mechanism that involves two conserved acidic residues, a putative proton donor at Glu225 and a nucleophile at Glu343 (Fig. 1).

Heparanase 2 (NP_068600) is produced by the HPSE2 gene (11 exons; human chromosome 10). Although less well characterized, heparanase 2 is well-conserved with forty-six orthologues (Ensembl version 57) from bony fish to human, and is 44% identical to heparanase. HPSE2 is not expressed in the eye (NEIBank) in keeping with an expression pattern generally distinct from HPSE. Amino acid length, predicted molecular weight and charge of the four RefSeq isoforms are: 1) 592, 66.6 kDa and pI 10; 2) 534, 60.1 kDa and pI 10; 3) 480, 53.9 kDa and pI 10.1; and 4) 540, 61.8 kDa and pI 9.9. N-linked glycosylation sites are predicted (NetNGlyc 1.0), but no O-glycosylation is predicted above threshold (NetOGlyc 3.1). Aceview suggests more isoforms.

2. Function

Heparanase displays enzymatic and non-enzymatic activities. The enzyme specifically cleaves the glycol-bond between D-glucuronic acid and N-sulfo glucosamine units leaving 4 - 5 kDa stubs attached to the core protein. Stubs appear to contribute to the binding site of prosecretory mitogen lacritin on syndecan-1 (Ma et al, 2006) that otherwise does not bind heparin. Lacritin is a constituent of the ocular tear film that flows over human corneal epithelial cells decorated with syndecan-1 (Ma et al, 2006). Other growth factors bind to and are sequestered on heparan sulfate proteoglycans. Local liberation by heparanase stimulates morphogenesis, for example FGF10-dependent branching morphogenesis in the salivary gland. Cytokines and other heparan sulfate binding proteins are also locally disseminated into tissues by heparanase. Heparanase's non-enzymatic activities are primarily mediated by its C-terminus domain in a manner largely uncharacterized, triggering cell signaling. Several surface binding proteins have been identified including heparan sulfate proteoglycans (for example syndecan-1), low density receptor-related protein-1 (LRP1) and the 300 kDa mannose-6-phosphate receptor, known as the insulin-like growth factor 2 receptor (IGF2R). Heparanase/MAPK1 signaling up regulates MMP9 and uPAR causing temporary loss of cell surface syndecan-1. Heparanase also activates AKT, P38, RAC1, PKC, and Src, thereby stimulating syndecan clustering, cell adhesion and tumorigenicity (Fux et al, 2009). Enzymatically active and inactive heparanase were noted to activate EGFR and to induce VEGF-A and VEGF-C expression via the Src pathway, providing, among other mechanisms, a molecular basis for the proangiogenic and protumorigenic capacity of heparanase (Fux et al, 2009). Mice overexpressing heparanase display elevated hematopoietic stem cell proliferation and epidermal stem cell migration, yet HPSE knockout mice are grossly normal with elevated MMP2 and MMP4 that may partially compensate. Knockout of the HPSE gene in mice has proved that there is only one gene encoding for heparanase with endo-glucuronidase activity, further indicating that HPSE2 lacks enzymatic activity.

3. Disease Involvement

Accumulation of compelling evidence implies that the enzyme is upregulated in primary human tumors and inversely correlates with survival rate of cancer patients post-operation. (Fux et al, 2009). Expression of HPSE is, for example, elevated 3 - 10 fold in breast and bladder cancer, and in glioblastoma, leukemia, myeloma, melanoma and sarcoma (Oncomine 4.3). Chronically elevated heparanase augments heparan sulfate 6-O-sulfation of syndecan-1, increases affinity for FGF1 and 2, and associates with tumor angiogenesis and metastatic potential. HPSE2, however, is often downregulated in cancer (Oncomine 4.3). The phosphosulfomannan PI-88 exhibits antiangiogenic, anti-metastatic and anti-restenotic activities and is the first heparanase-inhibiting compound subjected to phase II/III clinical trials in cancer patients. A non-

anticoagulant chemically modified heparin which is 100% N-acetylated and 25% glycol-split specifically inhibits heparanase activity and profoundly suppresses the progression of tumor xenografts produced by myeloma cells, indicating that heparanase enzymatic activity not only facilitates tumor metastasis, but also causally promotes the progression of primary tumors (Fux et al, 2009). Heparanase is upregulated in the colonic epithelium of patients with inflammatory bowel disease and heparanase enzymatic activity is markedly elevated in synovial fluid from rheumatoid arthritis patients, suggesting an important role for heparanase in inflammatory disorders. Ocular inflammation increases stromal heparanase from invading T-lymphocytes (Berk et al, 2004). Heparanase is also preferentially over-expressed in diabetic kidney and plays an important role in the pathogenesis of diabetic nephropathy, ultimately associated with kidney dysfunction (unpublished results).

4. Future Studies

Awareness to possible malfunction of the heparanase ‘off/on’ switch required for lacritin function deserves attention as the molecular bases for dry eye are dissected. Tear heparanase levels might be regulated in part by tear nucleotides, and via a different mechanism by androgens. ATP ligation of P2Y1 receptors stimulates secretion of activated heparanase in HPSE-transfected HEK-293 cells and in bovine corneal endothelial cells. Interestingly, a UTP agonist of the ocular surface P2Y2 receptor is in clinical trials for the treatment of dry eye. HPSE expression is substantially testosterone-responsive in mouse lacrimal and submandibular glands, but not in meibomian gland (GDS1361). Androgen therapy is also in clinical trials for dry eye. Heparanase is regarded as a valid target for drug development and a promising marker of several pathologies. Inhibitors of heparanase enzymatic activity are being developed, and new strategies are needed for the development of inhibitors against its non-enzymatic functions. Heparanase crystal structure will help, particularly in the context of its receptor(s). Development of effective inhibitory molecules and neutralizing antibodies will pave the way for advanced clinical trials in patients with cancer and other diseases involving heparanase (i.e., colitis, diabetic nephropathy). Understanding of heparanase signaling and elucidation of its route and function in the cell nucleus will further advance the field of heparanase research and its significance in health and disease.

Acknowledgments

Supported by NIH RO1 EY13143 and EY018222 to GWL, and NIH RO1 CA106456 to IV.

Abreviations

LACRT	lacritin
SDC1	syndecan-1
HPSE	heparanase

References

Barash U, Cohen-Kaplan V, Arvatz G, Gingis-Velitski S, Levy-Adam F, Nativ O, Shemesh R, Ayalon-Sofer M, Ilan N, Vlodavsky I. A novel human heparanase splice variant, T5, endowed with protumorigenic characteristics. FASEB J 2010;24:1239–1248. [PubMed: 20007507]

Berk RS, Dong Z, Alousi S, Kosir MA, Wang Y, Vlodavsky I. Murine ocular heparanase expression before and during infection with Pseudomonas aeruginosa. Invest. Ophthalmol Vis Sci 2004;45:1182–1187. [PubMed: 15037586]

Fux L, Ilan N, Sanderson RD, Vlodavsky I. Heparanase: busy at the cell surface. Trends Biochem Sci 2009;34:511–519. [PubMed: 19733083]

Ma P, Beck SL, Raab RW, McKown RL, Coffman GL, Utani A, Chirico WJ, Rapraeger AC, Laurie GW. Heparanase deglycanation of syndecan-1 is required for binding of the epithelial-restricted prosecretory mitogen lacritin. J. Cell Biol 2006;74:1097–1106. [PubMed: 16982797]

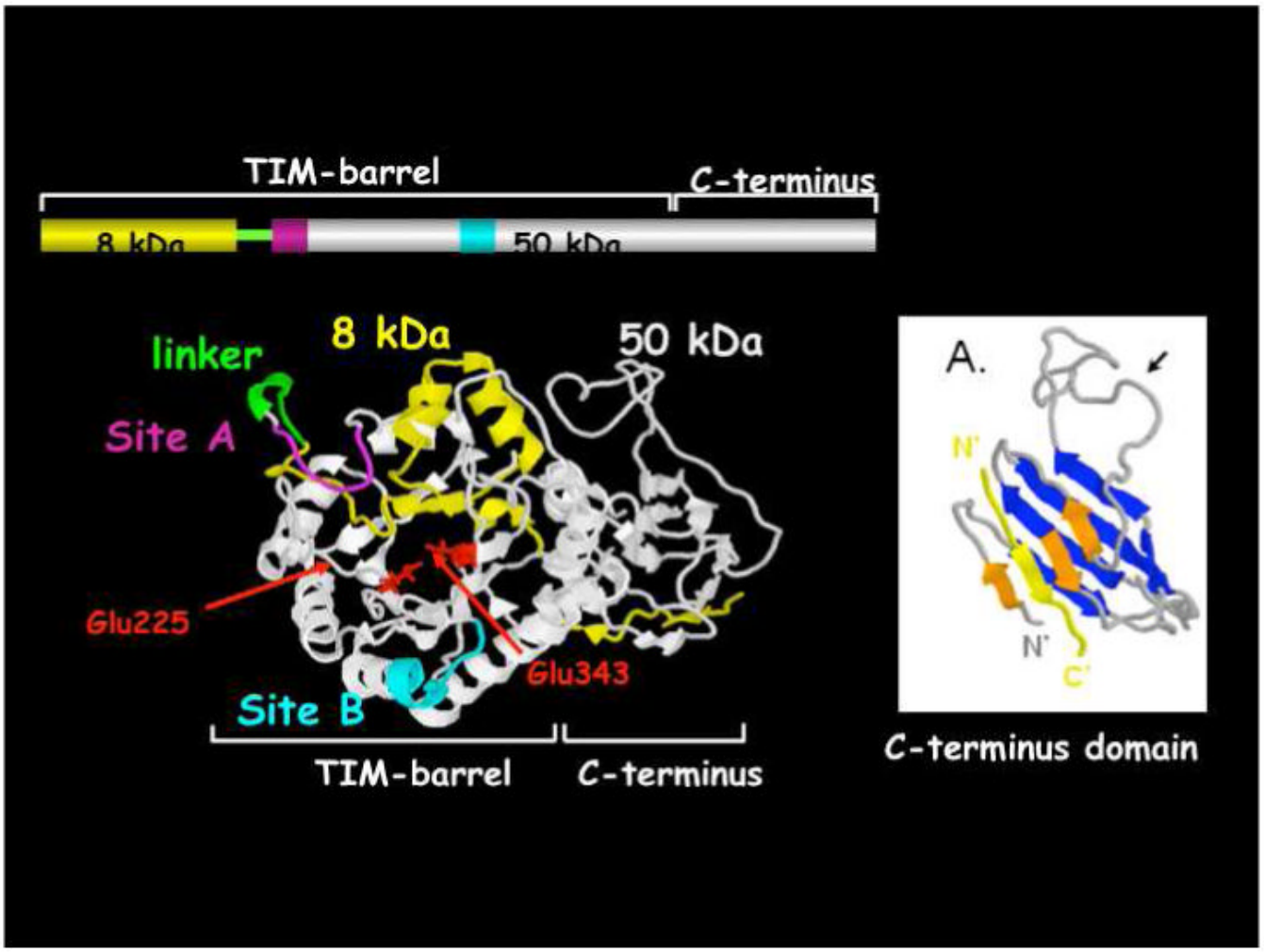

Fig. 1.

Predicted model of the 50+8 kDa heparanase heterodimer. Above, linear diagram of heparanase with uncleaved linker (green). Below left, predicted TIM-Barrel and C-terminal domains, enzymatically active site (E225 & E343, red), and heparin binding domains (Site A and B; purple & cyan). The linker (green) is shown uncleaved. Below right, isolated C-terminus with contribution from a strand of the 8 kDa fragment (yellow). A total of eight beta strands are formed. The arrow points to a flexible loop.