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COMMENTARY

# Heparanase and cancer progression: New directions, new promises

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**ABSTRACT**

Heparanase, the sole heparan sulfate degrading endoglycosidase, regulates multiple biological activities that enhance tumor growth, angiogenesis and metastasis. Much of the impact of heparanase on tumor progression is related to its function in mediating tumor-host crosstalk, priming the tumor microenvironment to better support tumor progression. Heparanase expression is enhanced in almost all cancers examined including various carcinomas, sarcomas and hematological malignancies. Numerous clinical association studies have consistently demonstrated that upregulated heparanase expression correlates with increased tumor size, tumor angiogenesis, enhanced metastasis and poor prognosis. Notably, heparanase is ranked among the most frequently recognized tumor antigens in patients with pancreatic, colorectal or breast cancer, favoring heparanase-based immunotherapy. Development of heparanase inhibitors focused on carbohydrate-based compounds of which 4 are being evaluated in clinical trials for various types of cancer, including myeloma, pancreatic carcinoma and hepatocellular carcinoma. Owing to their heparin-like nature, these compounds may exert off target effects. Newly generated heparanase neutralizing monoclonal antibodies profoundly attenuated myeloma and lymphoma tumor growth and dissemination in preclinical models, likely by targeting heparanase in the tumor microenvironment.

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## Heparanase and cancer

Heparan sulfate (HS) proteoglycans (HSPGs) are ubiquitous macromolecules associated with the cell surface and extracellular matrix (ECM) of a wide range of tissues.[1] The HS chains bind to and assemble ECM proteins, thus playing important roles in ECM integrity, barrier function and cell-ECM interactions.[1] HSPGs not only provide a storage depot for heparin-binding molecules (i.e., growth factors, chemokines, enzymes) in the tumor microenvironment, but also decisively regulate their accessibility, function and mode of action. It is therefore not surprising that a HS degrading enzyme (i.e., heparanase) is critically involved in tumor growth, angiogenesis and metastasis. Mammalian cells express a single dominant functional heparanase, an endoglycosidase that cleaves HS, leading to disassembly of the ECM and release of HS-bound bioactive molecules, thereby affecting tumor progression, angiogenesis and inflammation.[2-4] The heparanase mRNA encodes a 65 kDa pro-enzyme that is cleaved by cathepsin L into 8 and 50 kDa subunits that non-covalently associate to form the active enzyme.[5] Heparanase is up-regulated in essentially all human tumors examined, most often associating with reduced patients' survival post operation, increased tumor metastasis and higher vessel density.[2,6,7] A causal role of heparanase in tumor metastasis was demonstrated by the increased lung, liver and bone colonization of cancer cells following over-expression of the heparanase gene, and by a marked decrease in the metastatic potential of cells subjected to heparanase gene silencing.[8] Recent studies emphasize the involvement of heparanase in exosome formation,[9] activation of the immune system,[10,11] autophagy[12] and chemo-resistance,[12,13] further highlighting its significance in mediating the crosstalk between tumor cells and the tumor microenvironment and in dictating the tumor response to stress and host factors. The pro-tumorigenic effect of heparanase is attributed primarily to its HS degrading activity, facilitating cell invasion and 'priming' the tumor microenvironment. This notion is reinforced by *in vivo* studies indicating a marked inhibition of tumor growth in mice treated with heparanase-inhibiting heparin-like compounds (i.e., Roneparstat = SST0001, Necuparanib = M402, PI-88 = Mupafostat, PG545) now in phase I/II clinical trial in cancer patients.[14] In addition, enzymatically inactive heparanase promotes signal transduction, including Akt, STAT, Src, Erk and EGF-receptor phosphorylation,[15,16] highlighting the notion that non-enzymatic activities of heparanase may play a significant role in heparanase-driven tumor progression. Moreover, heparanase expression by tumor cells leads to upregulation of multiple genes (i.e., VEGF, HGF, RANKL, MMP-9, Tissue factor) that promote aggressive tumor behavior.[2,15,17] Altogether, it appears that heparanase is a master regulator of the aggressive phenotype of cancer, an important contributor to the poor outcome of cancer patients and a prime target for therapy.

## Heparanase neutralizing antibodies

As noted above, 4 carbohydrate-based heparanase inhibitors have reached clinical trials. These compounds apparently work

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by binding to the heparin/HS-substrate binding domain of the enzyme, thus blocking its accessibility to natural HS substrates. Owing to their heparin-based nature, these compounds can bind, in addition, to many heparin-binding proteins in vivo (which could be good or bad) leaving open the question as to how much of their anti-tumor effect is due specifically to blocking heparanase activity. Monoclonal antibodies against cancer related targets have met with considerable success due to their specificity and long half-life in humans, yet none have been tested clinically against heparanase. In previous studies we have identified 3 potential heparin-binding domains of heparanase.[18] Particular attention was given to the $Lys_{158}$-$Asp_{171}$ heparin binding domain (designated HBD1) since a peptide corresponding to this sequence physically interacts with heparin and HS with high affinity and inhibits heparanase enzymatic activity.[18] We have followed this rationale and generated a panel of monoclonal antibodies (mAbs) attempting to target the interaction of heparanase with its HS substrate. Our recent PNAS paper focuses on 2 mAbs (9E8, H1023) that neutralize heparanase enzymatic activity.[19] Moreover, both antibodies also substantially decreased the cellular uptake of latent heparanase, a HS-dependent mechanism that limits extracellular retention of the enzyme and thereby enables intracellular processing of the latent enzyme into its active form.[19] Thus, the newly generated antibodies not only neutralize the enzyme extracellularly, but also diminish heparanase levels inside the cell.

Both the 9E8 and H1023 mAbs markedly inhibited cellular invasion and tumor metastasis, the hallmarks of heparanase function. Moreover, both mAbs inhibited the spontaneous metastasis of ESb lymphoma cells from the subcutaneous primary lesion to the liver.[19] Importantly, treatment with mAb 9E8 or mAb H1023, as a single agent, attenuated the growth of human CAG myeloma and Raji lymphoma tumors, and even greater inhibition was observed by combining the 2 mAbs together (Fig. 1),[19] in agreement with the notion that combining 2 different mAbs increases the inhibitory outcome. Not surprisingly, mAb 9E8, or mAb H1023 are not cytotoxic to lymphoma, glioma, myeloma or breast carcinoma cells. This implies that the mAbs do not exert a direct effect on tumor cells but rather affect the tumor microenvironment. This is best demonstrated in Raji cells that lack intrinsic heparanase activity whereas tumor xenografts produced by these cells exhibit typical heparanase activity.[19] Thus the ability of mAbs 9E8 and H1023 to attenuate the growth of these tumors is due to neutralization of heparanase contributed by the tumor microenvironment. Importantly, evidence accumulating in recent years shows that targeting the tumor microenvironment (i.e., VEGF), may unexpectedly result in accelerated metastasis and more aggressive disease.[20] In contrast, our results indicate that heparanase targeting uniquely inhibits both tumor growth and metastasis, thus offering new opportunities and a safer mode to obstruct the tumor microenvironment.

## Heparanase and cancer immunotherapy

The novel heparanase-neutralizing mAbs described above are expected to exert high specificity, enabling solely the targeting of heparanase enzymatic activity and hence revealing its involvement and therapeutic significance in tumor progression as well as other pathologies, including inflammation.[21] The last decade critically revealed the decisive role of the tumor microenvironment, and more specifically inflammatory responses, in different stages of tumor development and metastasis.[22] The presence of inflammatory cells in the tumor mass has recently turned beneficial, due to the ability to re-direct memory T-cells against cancer cells, a notion that has met with tremendous clinical success.[23]

Interestingly, heparanase was ranked among the most frequently recognized tumor antigens in patients with pancreatic, colorectal or breast cancer.[24,25] Importantly, while causing the generation of high frequencies of specific CD4 and CD8 memory T cells, heparanase did not induce spontaneous regulatory T cell responses in cancer patients.[26] Owing to the absence of T-suppressor cells, anti-heparanase immunotherapy is expected to be prolonged and more efficient than that induced by other tumor associated antigens (TAAs). Chen et al selected 5 predicted epitopes and demonstrated cytotoxic T lymphocytes (CTL) responses

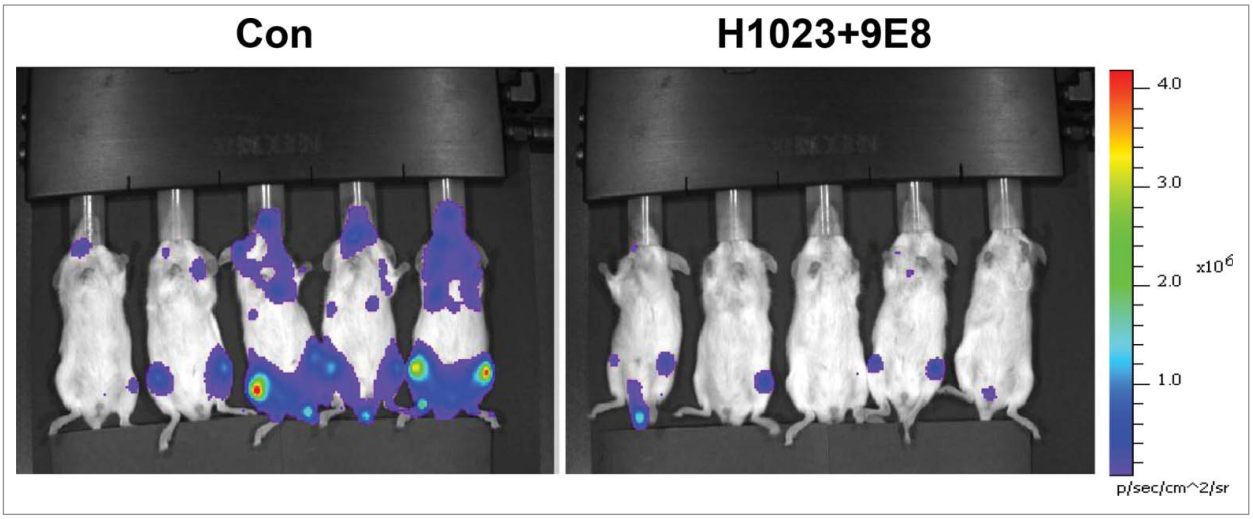


**Figure 1.** NOD/SCID mice (n = 5) were inoculated (iv) with luciferase labeled Raji- Burkitt's lymphoma cells. Mice were untreated (Con) or treated with mAb 9E8 or mAb H1023 (400 $\mu$g/mouse every other day) as single agents (not shown), or both together. Tumor growth was evaluated and quantified by IVIS imaging.

that were specific for heparanase-positive tumor cells.[27] Importantly, peptides derived from the mouse heparanase enzyme offered the possibility of not only immunizing against tumors, but also treating tumor-bearing hosts successfully.[25] In related studies, Zhang et al. applied a multiple antigen peptides (MAP) strategy and demonstrated that MAPs containing B cell epitope peptides derived from the human heparanase protein are capable of inducing a high titer of neutralizing antibodies in sera, indicating the feasibility of using MAPs to improve the immunogenicity of peptide vaccines targeting heparanase.[25] Using an endoplasmic reticulum retrieval signal, Zhou et al. designed heparanase epitope vaccine and reported that vaccination with dendritic cells pulsed with the modified peptide elicited a robust, specific CTL response.[28] The vaccine also significantly inhibited tumor growth and prolonged the lifespan of experimental mice indicating that this strategy could be used to improve the immunogenicity of heparanase CTL epitope peptides.[28] The above described considerations support the use of heparanase-based immunotherapy in combination with heparanase inhibitors and/or cytotoxic drugs.[25]

In a different set of experiments, it was recently reported that in contrast to freshly isolated T lymphocytes, heparanase is downregulated during *in vitro* - expanded T cells. Consequently, CAR-T cells engineered to express heparanase showed improved capacity to degrade the ECM, which promoted tumor T cell infiltration and antitumor activity.[29] The use of this strategy may enhance the antitumor activity of CAR-redirected T cells in individuals with stroma-rich solid tumors. Thus, while heparanase promotes tumor initiation, growth, and chemoresistance and is therefore considered a valid target for anti-cancer drugs, it can also be exploited to direct cytotoxic T-cells to attack tumors and to initiate anti-cancer immune responses.[29]

## Concluding remarks

While the involvement of heparanase in growth and metastasis of solid tumors (i.e., carcinomas and sarcomas) is well documented, its function in hematological malignancies (except myeloma) was not investigated in depth. Our study provides evidence that heparanase is expressed by human follicular and diffused non-Hodgkin's B-lymphomas, and, moreover, that heparanase inhibitors restrain the growth and dissemination of tumor xenografts produced by human lymphoma cells, likely by targeting heparanase in the tumor microenvironment.[19] Importantly, there is only a single enzymatically active form of heparanase and its inhibition is associated with little or no side effects. Notably, the crystal structure of the heparanase protein has recently been resolved,[30] promoting rational design of structure-based heparanase-inhibiting small molecules. These together with the existing compounds and the newly developed heparanase neutralizing antibodies will be applied in combination with approved therapies for the treatment of cancer, inflammation and other heparanase mediated disorders.

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No potential conflicts of interest were disclosed.

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