Commentary

Six blind men and the elephant—the many faces of heparan sulfate

Ajit Varki*

Glycobiology Research and Training Center, Departments of Medicine and Cellular and Molecular Medicine, University of California at San Diego, La Jolla, CA 92093-0687

It was six men of Indostan
To learning much inclined,
Who went to see the Elephant
(Though all of them were blind),
That each by observation
Might satisfy his mind. . . .
—John Godfrey Saxe, based on an Indian fable

Did you hear the one about the chemist, the biochemist, the molecular biologist, the physiologist, the physician-scientist, and the clinical oncologist? They went out to learn about a mysterious and complex creature called a heparan sulfate (HS) proteoglycan. Their interest was piqued by an intriguing paper in this issue of PNAS (1) with the title "Tumor Cell Surface Heparan Sulfate as Cryptic Promoters or Inhibitors of Tumor Growth and Metastasis." They came upon a knowledgeable female scientist who told them that HS chains belonged to a class of long acidic sugar chains called glycosaminoglycans (GAGs), which usually are attached to cell surfaces via a core protein (2). The combination of one or more HS chains and a core protein is called an HS proteoglycan (Fig. 1).

All six men felt they understood the primary observations in the paper. First, when mice carrying malignant tumors were injected with cloned purified bacterial enzymes called heparanases (also called heparinases or heparin lyases) that cleaved HS chains internally, the biological outcome depended on the type of enzyme used. Heparanase (Hep)-I induced significant acceleration of tumor growth, whereas Hep-III caused a reduction. The latter also seemed to limit the ability of the tumor to spread (metastasize) to other parts of the body. Second, when tumor cells were pretreated with enzymes before their i.v. injection, the degree of lung colonization again was correlated with the type of heparanase used. This finding in turn apparently correlated with changes in the ability of treated cells to invade a semiartificial basement membrane *in vitro*. Third, when tumor cells were treated with heparanases and the released HS fragments were purified and i.v.-injected, they recapitulated the *in vivo* effects of the enzymes. In keeping with this and with known differences in substrate specificity (3), the chemical structure of the HS fragments released by each enzyme was different. Fourth, histological analysis of the tumors in the mice injected with Hep-I showed reduced programmed cell death (apoptosis), increased cell growth, and enhanced recruitment of blood supply (angiogenesis), whereas Hep-III gave the converse effects. Thus, the authors suggested that the HS fragments generated by the enzymes were having effects on both the tumor cells and the endothelial cells lining their blood supply. Finally, both the enzymes and HS fragments had significant effects on certain growth factor-related intracellular signaling pathways, both on cultured cells and *in vivo* tumors. The authors concluded that HS chains on the surface of tumor cells contain both "activatory" and "inhibitory" sequences that were "in balance" and that these sequences could be differentially released by specific HS-degrading enzymes.

All six men agreed that the paper reported many interesting phenomena. However, each had their own unique perspectives derived from their respective scientific backgrounds and practical experiences. The chemist was fascinated by this striking demonstration of the power of chemical specificity in the HS fragments and its potential for practical applications. He was also proud of the fact that this biological work had originated from a lab with a strong record in chemical sciences. He thought that once the structural details were known, these HS fragments could be synthesized chemically and possibly used to treat humans with cancer.

The biochemist was reminded that HS chains were linear polysaccharides with complex and variable degrees of sulfation and epimerization, with such modifications occurring mostly in "blocks" along the length of the HS chains (Fig. 1; refs. 2 and 4). He wanted to know more about the substrate specificities of the heparanases. The female scientist told him that Hep-I cleaved HS chains within the modified block regions, whereas Hep-III had the converse specificity, cleaving mostly in between the blocks (Fig. 1; ref. 3). Thus, enzyme substrate specificity dictated whether the enzymes promoted or inhibited tumor growth and metastasis. The biochemist had heard that the HS chains were binding sites for certain growth factors (5). However, he was surprised to hear that the list of biologically relevant HS–protein interactions had become large and diverse (Table 1) and that several were now known to have specificity in binding to particular modified HS sequences (4). Overall he felt that this was an elegant demonstration of the differential substrate specificity of the two enzymes but was not too surprised that they had all these powerful biological activities on tumor growth and cell signaling. He also surmised that because almost every cell in the body seemed to have cell-surface HS proteoglycans, the effects of injected enzymes might be quite complex. Likewise, although the studies of fibroblast growth factor signaling were interesting, he felt that other biochemical pathways must have been affected.

The molecular biologist was not as impressed. He had noticed an increasing number of recent articles with titles claiming all sorts of biological effects for HS proteoglycans but was unable to get his arms around all of the sugar chemistry and jargon that seemed to be involved. He wondered how this fuzzy system without the digital elegance of the DNA-RNA-protein paradigm could have enough specificity to be so important biologically. He even began to suggest that these phenomena might be caused by some sort of "charge effect" of these acidic polysaccharides. He then was reminded by the female scientist that HS-chains actually are synthesized by a highly ordered sequence of enzymatic events in the Golgi apparatus, catalyzed by a large array of gene products that have an ancient evolutionary history,

See companion article on page 568.

*E-mail: avarki@ucsd.edu.

COMMENTARY

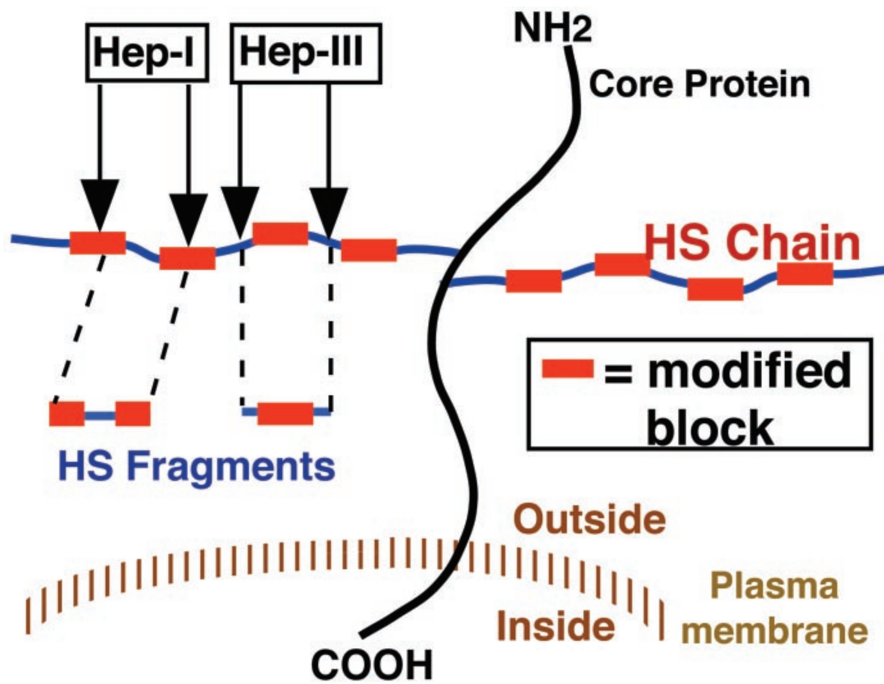

Fig. 1. Diagram of a typical HS proteoglycan with its core protein and HS chains. Heparanases (heparin lyases)-I and -III have different specificities in cleaving HS chains (inside and outside the modified blocks of HS chains, respectively), thus generating different kinds of HS-GAG fragments.

and are highly conserved (4). He immediately saw that this problem could be reduced to a more familiar genetic context and said he would be more convinced if this differential behavior of tumors could be reproduced in mice with different underlying genetic mutations of these HS synthesizing Golgi enzymes.

The physiologist thought it was all very interesting but felt that the effects of injecting an enzyme into a whole animal would be interpreted better by taking into account the topology of the vasculature and lymphatics. After all, the injected enzymes were proteins that could not diffuse freely through blood vessel walls. Thus, they should end up primarily in the bloodstream, either because they were injected directly into a vein or via lymphatic flow from the site of s.c. injections. After i.v. injection, the primary targets of the enzymes therefore should be blood cells and endothelial cells. With s.c. injection, there would be additional interactions with cells in the draining lymphatics and lymph nodes before the enzymes finally reached the bloodstream. In either case, the primary targets likely would not be the tumor cells growing in the flank of the animal. Thus, the HS fragments circulating in the intact animal should have originated from many cell types, not just the tumor cells. Indeed, they might not even be the same in structure as the ones released from the tumor cells *in vitro*. He also was interested to know what might happen if HS fragments circulating in the blood of a treated mouse could be isolated and injected back into another mouse with a tumor. Finally, he wondered about the practical value of this approach, because the immune system would likely make antibodies against these bacterial enzymes, making it difficult to administer them on multiple occasions.

The physician-scientist was impressed by the dramatic differences in tumor behavior resulting from the injection of the two enzymes. He was not as concerned with the complexity of the *in vivo* studies. He knew that his basic science colleagues had a dim view of experiments in whole animals unless they were very tightly controlled or based on precise genetic manipulations. Although he generally agreed with this reductionist approach, he also realized that if basic findings were ever to be translated in clinical practice, it was necessary to do some "dirty" whole-animal experiments and pursue the ones producing such dramatic results with more precisely interpretable studies. However, he wondered why other well described effects of HS chains on tumor cell biology had not been considered. For example, he had seen reports that HS chains could modulate many other biological pathways thought to be relevant to tumor behavior including blood coagulation (6), cell adhesion by molecules called selectins (7), basement membrane degradation (8, 9), etc. Finally, he was concerned that potent bacterial products such as endotoxin could have complicated the picture, despite the single passage of the enzymes over an endotoxin removal column. If this approach were to be tried in humans, much more stringent approaches to eliminate endotoxin contamination would be needed.

The clinical oncologist was not going to be impressed by yet another report of "curing cancer in mice" unless it had some direct relevance for his patients dying of cancer. When he was told that HS chains were close cousins of a commonly used anticoagulant called heparin (10), he wondered whether there were any connections with papers he had seen concerning the beneficial effects of anticoagulation in human cancer (6). In fact, he had heard that someone was proposing that heparin treatment in cancer be revisited under a new paradigm of action (selectin inhibition; refs. 7 and 11). Regardless, he knew that very few of these successes in mouse cancer treatment ever made it into the clinic. Even if this one did, it would be many, many years after the initial basic science observations, too late for his patients who were dying of cancer right now. He sometimes wished that the system for translation of such findings to the bedside was not so rigorous and slow. After all, what did a patient with advanced cancer have to lose by trying out something that worked in mice? For the same reason, he

Table 1. Examples of molecules whose biological activity is modulated by binding to heparan sulfate chains*

Angiogenesis: Angiostatin, endostatin, vascular endothelial growth factors
Cell–matrix interactions: Laminin, fibronectin, thrombospondin, collagen types I, II, and V, fibrillin, tenascin, vitronectin
Coagulation/fibrinolysis: Antithrombin III, heparin cofactor II, tissue factor pathway inhibitor, thrombin, protein C inhibitor, tissue plasminogen activator, plasminogen activator inhibitor-1
Growth factors/morphogens: Fibroblast growth factors (FGFs) and FGF receptors, *Wingless* factors (Wnts), Hepatocyte growth factor (HGF, scatter factor), transforming growth factors (TGFs) β 1 and 2, bone morphogenic proteins (BMPs) 2, 3, 4, and 7, *Hedgehog* factors
Inflammation: Chemokines (e.g., MIP-1b); cytokines (e.g., IL-2, -3, -4, -5, -7, -8, and -12); L- and P-Selectins, Extracellular superoxide dismutase; antimicrobial peptides.
Lipid metabolism: Lipoprotein lipase, hepatic lipase, apoliprotein E

*Modified from ref. 4.

also was not too concerned about using bacterial enzymes in human therapy. Besides, there were clear precedents such as bacterial streptokinase for dissolving blood clots and L-asparaginase for childhood acute leukemia.

And so these men of Indostan
Disputed loud and long,
Each in his own opinion
Exceeding stiff and strong,
Though each was partly in the right,
And all were in the wrong!
—John Godfrey Saxe

At the end of the day, they all left with different opinions about HS chains, each thinking he knew what was right and wrong with the paper. However, they were all fascinated by the complexities of HS proteoglycans and their known and potential roles in so many areas of biology and medicine. Meanwhile, the glycobiologist who had been observing the proceedings and offering information about HS chains smiled to herself. She knew they were all right and yet all wrong and would never see the big picture until they were willing to learn many more details about the biosynthesis, structure, turnover, biology, and functions of HS proteoglycans. At the very least, she felt that they now might pay more attention when she talked to them about the biological importance of sugar chains. However, she also remembered a comparison that Kasai and Hirabayashi (12) had made between the "digital" world of DNA and the "analog" world of glycan chains, ". . . the contrast between Japanese culture, which is characterized by ambiguity and compromise, and European culture, which always demands clear-cut answers."

1. Liu, D., Shriver, Z., Venkataraman, G., El Shabrawi, Y. & Sasisekharan, R. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 568–573.
2. Esko, J. D. & Lindahl, U. (2001) *J. Clin. Invest.* **108,** 169–173.
3. Shriver, Z., Sundaram, M., Venkataraman, G., Fareed, A., Linhardt, R., Biemann, K. & Sasisekharan, R. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 10365–10370.
4. Esko, J. D. & Selleck, S. B. (2002) *Annu. Rev. Biochem.*, in press.
5. Faham, S., Hileman, R. E., Fromm, J. R., Linhardt, R. J. & Rees, D. C. (1996) *Science* **271,** 1116–1120.
6. Zacharski, L. R. & Ornstein, D. L. (1998) *Thromb. Haemostasis* **80,** 10–23.
7. Borsig, L., Wong, R., Feramisco, J., Nadeau, D. R., Varki, N. M. & Varki, A. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 3352–3357.
8. Vlodavsky, I. & Friedmann, Y. (2001) *J. Clin. Invest.* **108,** 341–347.
9. Parish, C. R., Freeman, C. & Hulett, M. D. (2001) *Biochim. Biophys. Acta* **1471,** M99–M108.
10. Hirsh, J. (1991) *N. Engl. J. Med.* **324,** 1565–1574.
11. Varki, N. & Varki, A. (2002) *Semin. Thromb. Hemostasis*, in press.
12. Kasai, K. & Hirabayashi, J. (1996) *J. Biochem. (Tokyo)* **119,** 1–8.