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Heparin: an intervenor in cell communication

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Abstract

It was nearly 100 years since heparin was discovered, but the role of this widely used anticoagulant is still remarkably thought provoking now. During pathological processes such as atherosclerosis, inflammation, cancer and infection, phenomena of cell adhesion are ubiquitous and complicated. Heparin exerts anti-adhesion activity appearing as a common mechanism of its potential polypharmacology in those diseases. Furthermore, heparin can bind a variety of signalling molecules such as growth factors, cell surface proteins of pathogens and most notably, cell adhesion molecules. These signalling molecules are involved in cell communication, acting as ligands, receptors and second messengers. Considering that heparan sulphate glycosaminoglycan is increasingly recognized as a key mediator in many cellular processes, the structural similarity with heparan sulphate suggests that heparin is a multifunctional intervenor in cell communication.

Keywords: heparin ● cell adhesion ● cell communication ● heparan sulphate ● heparanase

Introduction

Well known as an anticoagulant, pharmaceutical standard heparin is derived from natural animal sources such as pig intestine and beef lung. Originally from dog liver, heparin was discovered by McLean in 1916 and the term *heparin* was coined by Howell in 1918. The story of heparin discovery was fascinating considering McLean previously set out to look for natural procoagulant phosphatides [1]. Both McLean's heparphosphatide and Howell's heparin differ from today's heparin, but their discovery changed the classical viewpoint that anticoagulant in the body is protein based. Up to now, diverse new properties of heparin have been elucidated. The potential role of heparin in normal physiologic and pathophysical processes is analyzed in this review.

Effects of heparin beyond anticoagulant

Heparin used today is a mixture of water-soluble, highly sulphated and linear polysaccharides. As a member of the glycosaminoglycan family, heparin is mainly composed of repeating, variably-sulphated disaccharide units containing iduronic acid 2-sulphate, glucosamine 2, 6-disulphate and non-sulphated glucuronic acid. The specific pentasaccharide sequence of heparin contributes to its electrostatic interaction with antithrombin (AT), which then inactivates thrombin and other proteases involved in blood clotting [2]. Heparin remains the most important antithrombotic therapeutic drug until now. Actually, only one-third of heparin molecules contain pentasaccharide sequences with high affinity for AT [3]. The functional versatility and other therapeutic potential (Table 1) have been ascribed to heparin

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Table 1 Non-anticoagulant effects of heparin

Disease states sensitive to heparin	Effects of heparin
Asthma	Reduces cell activation and accumulation in airways, neutralizes mediators and cytotoxic cell products and improves lung function
Arthritis	Inhibits cell accumulation, collagen destruction and angiogenesis
Inflammatory bowel disease	Inhibits inflammatory cell transport
Cancer	Inhibits tumour growth, metastasis and angiogenesis and increases survival time
Infection	Reduces development of adhesions and promotes the removal of bacteria by the host defence
Osteoporosis	Reduces activation of osteoblasts
Atherosclerosis	Activates LPL
Transplant rejection	Modulates immunity

beyond its role as an anticoagulant [4]. Anti-inflammatory effects of heparin have been described frequently. Heparin has been shown to benefit patients with allergic inflammation, bronchial asthma and ulcerative colitis [5]. After the asthma patients inhaled aerosolized heparin, no change is detected in the level of coagulation. Heparin proved to be more effective than cromolyn sodium [6]. As the classic anticoagulant of choice to treat cancer patients with thrombosis, heparin also can improve cancer survival rates independently of the effect on thromboembolism. Heparin mainly prevents the metastatic cascade, which is responsible for most cancer-related deaths. Chemically modified heparins with no or limited anticoagulant activity also showed anti-metastatic properties [7]. LHD, one orally absorbable heparin derivative dramatically attenuated metastasis experimentally induced by murine melanoma or human lung carcinoma cells. Furthermore, it prevented tumour growth in secondary organs [8].

Non-anticoagulant heparins

The reason to develop non-anticoagulant heparin is the risk of bleeding. Considerable effort has been made towards the discovery of non-anticoagulant heparins including "heparin-like" derivatives and heparin mimicking polyanions. The non-anticoagulant heparins actually represent a certain aspect of heparin application. Various non-anticoagulants are helpful to further understand chemistry and biology of heparin. Non-anticoagulant heparins have clinical potential because they can be administered at a higher dose. An N-acetylated, glycol-split heparin provides an example of application of a non-anticoagulant heparin that inhibits

cancer in animal models without unwanted side effects [9]. Recently, methods have been described to prepare structure-optimized heparin derivatives to increase selectivity [10].

Low molecular weight heparins (LMWHs) have molecular weights ranging from 2000 to 10,000 daltons with peak frequencies of 4000–6000 daltons. They are derived from unfractionated heparin via chemical or enzymatic methods, such as: nitrous acid depolymerization (dalteparin, nadroparin, reviparin), enzymatic degradation (tinzaparin) or benzylation followed by alkaline hydrolysis (enoxaparin) [11]. LMWH may have several advantages over unfractionated heparin: it has a more predictable anticoagulant effect with, requires no monitoring of anticoagulation and has a lower incidence of heparin-induced thrombocytopenia (HIT) [12].

Cells adhere to communicate

Cell adhesion is involved in most physiological cell functions and pathological situations such as metastasis formation, tissue invasion by pathogens, atherosclerosis, inflammation or host-biomaterial interaction [13]. Since cells are not often found in isolation, they interact with other cells or non-cellular components of their environment. Cell adhesion is ubiquitous and not simply a biomechanical process for "gluing" cells together [14]. The importance of cell adhesion is concerned with cell communication. When adhering, signals pass between the cells [15]. The cells that are not immediately adjacent indirectly communicate by releasing chemical messengers called ligands. The plasma-membrane proteins receiving information are known as receptors. A signalling pathway starts when a signalling ligand activates its receptor. Usually, second messengers such as cyclic AMP (cAMP) and Ca^{2+} participate in signalling pathways. Cell adhesion molecules (CAMs), termed 'receptors', are involved in a variety of signalling events and have the ability to initiate the formation of scaffolds that permit the efficient flow of information in signalling pathways. Defects in cell adhesion are usually attributable to defects in expression of adhesion molecules. Several cell adhesion molecules, such as integrins, have been implicated in the formation of complexes that are composed of extracellular ligands, tyrosine kinase receptors and cytoskeletal proteins [16]. It has become increasingly recognized that the endothelium is not simply a passive barrier, but a crucial player in maintaining vascular homeostasis and regulating the passage of materials between the blood and the vessel wall. Besides structural functions, cell adhesion molecules involved in endothelial cell–cell interaction play an important role in inducing and integrating intracellular signals that, in turn, impact on vascular cell physiology [17].

Anti-adhesion activity of heparin

Anti-adhesion activity appears as a common mechanism of non-anticoagulant effects of heparins. During inflammatory responses,

leukocyte-endothelial cell adhesion is an important event preceding leukocyte transendothelial migration from the circulation into tissues. There is accumulating evidences that heparin interferes with the adhesion of leukocytes to the endothelium. Pre-incubation of polymorphonuclear leucocytes (PMNs) with heparin followed by washing inhibited their adhesion to endothelial cells under different conditions of cellular activation. This implies that heparin can exert anti-adhesion effects even when not directly present in the system [18]. LMWH has been reported to inhibit both adhesion and transmigration of leucocytes to endothelial cells. Parnaparin, a LMWH, can prevent platelet activation and interaction with PMN [19]. In the initiation of ischemia/reperfusion (I/R)-mediated injury, heparin significantly reduces the hyperadhesiveness of neutrophils to endothelial cells and following transendothelial migration [20]. The formation of cancer cells-platelets emboli complexes facilitates metastases. With platelets aid, the cancer cells separate from the primary tumour, avoid from the cytotoxic activity of natural killer cells. Furthermore, platelets also assist cancer cells adhere to vascular endothelial cells, migrate across blood vessel walls into the bloodstream and disperse throughout the body to generate new colonies. Heparin may inhibit metastasis by blocking cancer cells-platelets interaction and cancer cells-endothelial cells interaction [21]. A critical step for infection is adhesion of pathogenic microbes to host cells, which can be inhibited by heparin. The adhesion of *E. coli* O157: H7 to human colonic epithelium can be blocked by heparin in a dose-dependent fashion [22]. Early administration of intravenous therapeutic dose heparin may be associated with decreased mortality when administered to patients diagnosed with septic shock [23].

Heparin and cell adhesion molecules

The counter-ligands for the selectins are carbohydrate moieties that appear to require an anionic charge for recognition. Heparin might serve as a potential antagonist of selectin ligand [24]. Despite no obvious structural similarity to the natural ligands of selectins, unfractionated heparin was shown to effectively inhibit P- and L-selectin binding to their natural ligands (sLex) [25]. Selectin-binding properties of heparins can be controlled by structural modifications [26]. The firm integrin-mediated adhesion, another major adhesion step after the initial selectin-mediated rolling, can also be inhibited by heparin. Mac-1, also known as complement receptor 3, is one of the most versatile adhesion molecules. Its interaction with intercellular adhesion molecule-1 (ICAM-1) mediates leukocyte adhesion on endothelial cells. Heparin binds to Mac-1 and inhibits Mac-1-mediated ligand binding [27]. HIV has an adhesion molecule termed gp120 that binds to its ligand CD_4 expressed on lymphocytes. Heparin inhibits the replication of HIV-1 *in vitro*, and the inhibition activity is correlated with the ability of the heparin to bind to gp120 [28]. The interactions of platelets with heparin are especially confusing. Heparin

promotes thrombosis and platelet activation due to the generation of an antibody to the heparin-platelet factor 4 complex [29]. HIT antibodies also have been demonstrated to induce platelet–leukocyte aggregates in a heparin-dependent interaction [30]. Heparin was found to directly bind to platelet integrin alpha(IIb) beta(3) (glycoprotein IIb/IIIa) and enhance its binding of fibrinogen [31]. Apart from HIT immune reaction, the ability of heparin to inhibit thrombin-induced platelet activation was noted a long time ago. Platelets bind to matrix von Willebrand factor by surface glycoprotein Ib (Gp1b) receptors. Thrombin binds to Gp1b via its heparin-binding site and that heparin is able to interfere with the thrombin-Gp1b interaction. The extent of the inhibitory effect is related to the molecular weight of heparin fractions [32].

Heparin binds to messengers in cell communication

As reviewed [33], heparin has been shown to bind to a variety of biologically important proteins including enzymes, growth factors, extracellular matrix (ECM) proteins and the cell surface proteins of pathogens (Table 2). The activities of these proteins are regulated by heparin, when electrostatic forces between the positively charged amino acids and the polyanionic groups in the glycosaminoglycan chain effect changes in protein conformations. The cytokines, as first messengers, form a complex network of immune competent cells communication [34]. Heparin inhibits cytokines that may contribute to angiogenesis, in which the fibroblast growth factor (FGF) cytokine family is among the best understood. Both heparin and LMWH have been shown to inhibit FGF-induced angiogenesis in a human *in vitro* angiogenesis model [35]. Heparin can negatively modulate store operated Ca^{2+} channels and other non-capacitive Ca^{2+} channels [36]. Heparin also inhibits the Ca^{2+} release induced by inositol 1, 4, 5- trisphosphate (IP_3). The effect on Ca^{2+} release appeared specific for heparin and was not reproduced by other polysaccharides such as chondroitin sulphates [37]. Heparin can negatively modulate Ca^{2+} channels and these activities might also account for its multiple biological effects.

What makes cells sticky?

Compared with heparin, discovery of heparan sulphate (HS), originally as an impurity in heparin, was much more recent. HS are abundantly expressed at cell surfaces and in the ECM as part of proteoglycans. Over the past decade, the supposed functions of HS have increased dramatically, from being seen as simply structural determinants of the ECM, to being key players in the regulatory network of the cells. Because of their high negative charge, HS interacts with a wide variety of proteins such as growth factors, enzymes and ECM proteins. Binding HS provides a means to both concentrate

Table 2 Selected heparin-binding proteins

Heparin-binding protein		Biological roles
Proteases	AT	Anticoagulation and antithrombosis
	HCF II	Anticoagulation and antithrombosis
Growth factors and cytokines	FGF-1	Cell proliferation, differentiation, morphogenesis and angiogenesis
	FGF-2	Same as FGF-1
	VEGF	Cell growth, morphogenesis and development
	HGF	Haepatocyte regeneration, morphogenesis, cell motility, tumourigenesis and metastasis
	PF-4	Inflammation and wound healing
	IL-8	Pro-inflammatory cytokine
	γ-interferon	Dimerization and modulation of proteolytic processing
Enzymes	ApoE	Lipid transport, Alzheimer's disease risk factor
	Annexin V	Anticoagulant activity
	Heparanase	Inflammation and metastasis
Pathogen proteins	HIV-1 gp120	Viral entry
	Tat	Transactivating factor, primes cells for HIV infection
	HSV glycoproteinB and glycoproteinC	HSV attachment to the host cell
Adhesion proteins	Vitronectin	Cell adhesion and migration
	Fibronectin	Cell adhesion and traction
	Selectins	Adhesion, inflammation and metastasis
	Integrins	Cell adhesion and signalling

and restrict the diffusion of soluble proteins like growth factors. In addition, HS-binding by ECM components enhances the structural integrity of the tissue. In fact, almost every ECM molecule contains binding sites for HS, suggesting that the balance between adhesion and motility depends on the integration of signals mediated through proteoglycan-binding and integrin-based adhesion mechanisms [38]. Like an antenna on cell, HS play a crucial role in cell communication in responding to the diverse extracellular signals including the members of the FGF family and their receptor tyrosine kinases, transforming growth factors (TGFs), bone morphogenetic proteins (BMPs), Wnt proteins, chemokines and interleukins, as well as enzymes and enzyme inhibitors, lipases and apolipoproteins (LPLs), and ECM and plasma proteins [39]. Heparan sulphate proteoglycans (HSPGs) are believed to act as co-receptors that are capable of influencing cell fate by integrating cellular signals [40]. Besides interaction with endogenous factors, HS have also been demonstrated to act as receptors for a number of different pathogens. HS provides an easily accessible primary receptor for viral adhesion. This is followed by interaction of viral proteins with other cellular mediators for adhesion and penetration in the form of secondary receptors [41].

The noncovalent interactions of sulphated polysaccharides with proteins effect changes in protein conformation, facilitate protein–protein interaction, sequester proteins at the cell surface. Exogenous heparin, displaying higher N- and O-sulfation than HS, strongly competes with HS for binding proteins. This competitive activity can be reduced by selective desulfation [42]. Still, it is oversimplified to take heparin for differing HS with the higher negative charge density. Characterization of HS from cell lines has indicated that HS chains (unlike those of heparin) exhibit domain structure. HS stands out in glycosaminoglycans with the most highly variable structural motifs, which are primarily responsible for the numerous protein binding and regulatory properties [43]. Additionally, both heparin and HS contain a wide range of sequences. The relevance of HSPGs and heparin is very complicated and remains not fully certain. Many proteins that interact with HS are initially identified as having heparin-binding properties. Heparin and HS are also important in another aspect of HIV-1 infection. One of the proteins essential for HIV-1 replication is the Tat protein, which has the ability to enter cells and is believed to play a role in priming cells for infection. Tat is a heparin-binding protein, which also interacts with HS [44]. Interaction of the HS with LPL plays a role in lipoprotein

clearance from the plasma. Heparin is reported to bind LPL more strongly than HS. Exogenous heparin causes the release of cell surface-bound LPL into the plasma by competing with HSPG for the enzyme. The heparin-complexed LPL can then bind to circulating lipoproteins to catalyze the release of free fatty acids, thus 'clearing' them from the circulation [45]. There is now ample evidence that HS has an important role in many aspects of an inflammatory response, from the initial adhesion of leukocytes to the endothelium to the subsequent leukocyte extravasation and establishment of a chronic inflammatory response. The molecules that interfere with HS function have considerable potential as anti-inflammatory agents. In this regard, it has been known for many years that heparin has anti-inflammatory activity [46].

Heparin and heparanase

Heparanase, a hydrolase, is capable of cleaving HS and hence participates in degradation and remodelling of the ECM. Heparanase expression is rare in normal tissue, but is evident in activated inflammatory cells and most human cancer cells. At inflammatory sites, heparanase may synergize with other cellular factors to promote endothelial retraction and enhance leukocyte extravasation [47]. A critical event in the process of cancer invasion and metastasis is the degradation of various ECM components, which causes release of growth factors sequestered by HS chains, thus accelerating tumour growth and metastasis [48]. Inhibition of heparanase is a promising target for a novel strategy in cancer therapy. Heparin and some chemically modified heparins can act as heparanase inhibitors. The N-acetylated glycol-split species of heparin, as well as heparanase gene silencing, inhibit tumour metastasis, angiogenesis and inflammation in experimental animal models [49]. Effect of heparin on inhibiting graft rejection might involve modulating heparanase cell-surface expression or secretion, which impairs T lymphocytes from penetrating blood vessel walls, accumulates in target organs and mediates delayed-type hypersensitivity (DTH) reaction [50].

Conclusion

Further insight into how cells communicate is necessary for understanding numerous biological processes. For example, bacteria can use cell communication mediated by diffusible signal molecules to monitor their population density and to modulate their behaviours in response to their environment. It has been suggested blocking bacterial communication may be an alternative strategy to fight infection [51]. Heparin/HS participated in multifarious modulation in cell communication. This opens up a large number of new therapeutic applications for the old molecule in the treatment of cancer, viral and bacterial infections, various inflammatory diseases, atherosclerosis, wound healing and Alzheimer's disease. By focusing on the how protein and saccharide interact with one another, a library of heparin-inspired drugs can be designed with 'tailor-made' therapeutic activities. However, research in saccharide–protein interaction has lagged behind that of protein–nucleic acid or protein–protein interaction. The major limitation in utilizing heparin in new ways is that its high potency as an anticoagulant becomes a side effect. The next challenge for heparin/HS research may be the development of 'recombinant' heparin [52] with more precise structure so as to achieve high functional selectivity. In the coming years of the glycomics era, continuing efforts on in heparanome research [53] will likely lead to novel molecular therapeutic approaches for combating health problems.

Acknowledgements

References

1. **Marcum JA.** The origin of the dispute over the discovery of heparin. *J Hist Med Allied Sci.* 2000; 55: 37–66.
2. **Seyrek E, Dubin PL, Henriksen J.** Nonspecific electrostatic binding characteristics of the heparin-antihrombin interaction. *Biopolymers.* 2007; 86: 249–59.
3. **Nugent MA.** Heparin sequencing brings structure to the function of complex oligosaccharides. *Proc Natl Acad Sci USA.* 2000; 97: 10301–3.
4. **Lever R, Page CP.** Novel drug development opportunities for heparin. *Nat Rev Drug Discov.* 2002; 1: 140–8.

5. **Young E.** The anti-inflammatory effects of heparin and related compounds. *Thromb Res.* 2008; 122: 743–52.
6. **Diamant Z, Page CP.** Heparin and related molecules as a new treatment for asthma. *Pulm Pharmacol Ther.* 2000; 13: 1–4.
7. **Niers TM, Klerk CP, DiNisio M, et al.** Mechanisms of heparin induced anti-cancer activity in experimental cancer models. *Crit Rev Oncol Hematol.* 2007; 61: 195–207.
8. **Lee DY, Park K, Kim SK, et al.** Antimetastatic effect of an orally active heparin derivative on experimentally

induced metastasis. *Clin Cancer Res.* 2008; 14: 2841–9.
9. **Casu B, Vlodavsky I, Sanderson RD.** Non-anticoagulant heparins and inhibition of cancer. *Pathophysiol Haemost Thromb.* 2008; 36: 195–203.
10. **Huang L, Kerns RJ.** Diversity-oriented chemical modification of heparin: identification of charge-reduced N-acyl heparin derivatives having increased selectivity for heparin-binding proteins. *Bioorg Med Chem.* 2006; 14: 2300–13.
11. **Hirsh J, Anand SS, Halperin JL, et al.** Guide to anticoagulant therapy:

heparin—a statement for healthcare professionals from the American Heart Association. *Circulation.* 2001; 103: 2994–3018.

12. **Hirsh J, Raschke R.** Heparin and low-molecular-weight heparin: the seventh ACCP conference on antithrombotic and thrombolytic therapy. *Chest.* 2004; 126: 188S-203S.

13. **Hynes RO.** Cell adhesion: old and new questions. *Trends Cell Biol.* 1999; 9: 33–7.

14. **Braga V, Harwood AJ.** Super glue. *Nat Cell Biol.* 2001; 3: E168–70.

15. **Singer SJ.** Intercellular communication and cell-cell adhesion. *Science.* 1992; 255: 1671–7.

16. **Juliano RL.** Signal transduction by cell adhesion receptors and the cytoskeleton: functions of integrins, cadherins, selectins, and immnoglobulin- superfamily members. *Annu Rev Pharmacol Toxicol.* 2002; 42: 283–323.

17. **Liebner S, Cavallaro U, Dejana E.** The multiple languages of endothelial cell-to-cell communication. *Arterioscler Thromb Vasc Biol.* 2006; 26: 1431–8.

18. **Lever R, Hoult JR, Page CP.** The effects of heparin and related molecules upon the adhesion of human polymorphonuclear leucocytes to vascular endothelium *in vitro.* *Br J Pharmacol.* 2000; 129: 533–40.

19. **Maugeri N, Di-Fabio G, Barbanti M, *et al.*** Parnaparin, a low-molecular-weight heparin, prevents P-selectin dependent formation of platelet-leukocyte aggregates in human whole blood. *Thromb Haemost.* 2007; 97: 965–73.

20. **Kouretas PC, Kim YD, Cahill PA, *et al.*** Nonanticoagulant heparin prevents coronary endothelial dysfunction after brief ischemia-reperfusion injury in the dog. *Circulation.* 1999; 99: 1062–8.

21. **Smorenburg SM, Van Noorden CJ.** The complex effects of heparins on cancer progression and metastasis in experimental studies. *Parmacol Rev.* 2001; 53: 93–106.

22. **Gu L, Wang H, Guo YL, *et al.*** Heparin blocks the adhesion of *E. coli* O157:H7 to human colonic epithelial cells. *Biochem Biophys Res Commun.* 2008; 369: 1061–4.

23. **Zarychanski R, Doucette S, Fergusson D, *et al.*** Early intravenous unfractionated heparin and mortality in septic shock. *Crit Care Med.* 2008; 36: 2973–9.

24. **Borsig L.** Selectins facilitate carcinoma metastasis and heparin can prevent them. *Physiology.* 2004; 19:16–21.

25. **Wang L, Brown JR, Varki A, *et al.*** Heparin's anti-inflammatory effects require glucosamine 6-O-sulfation and aremedi-

ated by blockade of L- and P-selectins. *J Clin Invest.* 2002; 110: 127–36.

26. **Wei M, Tai G, Gao Y, *et al.*** Modified heparin inhibits P-selectin-mediated cell adhesion of human colon carcinoma cells to immobilized platelets under dynamic flow conditions. *J Biol Chem.* 2004; 279: 29202–10.

27. **Peter K, Schwarz M, Conradt C, *et al.*** Heparin inhibits ligand binding to the leukocyte integrin Mac-1 (CD11b/CD18). *Circulation.* 1999; 100: 1533–9.

28. **Rider CC.** The potential for heparin and its derivatives in the therapy and prevention of HIV-1 infection. *Glycoconj J.* 1997; 14: 639–42.

29. **Jang IK, Hursting MJ.** When heparins promote thrombosis: review of heparin-Induced thrombocytopenia. *Circulation.* 2005; 111; 2671–83.

30. **Khairy M, Lasne D, Amelot A, *et al.*** Polymorphonuclear leukocyte and monocyte activation induced by plasma from patients with heparin-induced thrombocytopenia in whole blood. *Thromb Haemost.* 2004; 92: 1411–9.

31. **Sobel M, Fish WR, Toma N, *et al.*** Heparin modulates integrin function in human platelets. *J Vasc Surg.* 2001; 33: 587–95.

32. **De Candia E, De Cristofaro R, Landolfi R.** Thrombin-induced platelet activation is inhibited by high- and low-molecular-weight heparin. *Circulation.* 1999; 99: 3308–14.

33. **Capila I, Linhardt RJ.** Heparin-protein interactions. *Angew Chem Int Ed.* 2002; 41: 390–412.

34. **Forsten-Williams K, Chu CL, Fannon M, *et al.*** Control of growth factor networks by heparan sulfate proteoglycans. *Ann Biomed Eng.* 2008; 36: 2134–48.

35. **Collen A, Smorenburg SM, Peters E, *et al.*** Unfractionated and low molecular weight heparin affect fibrin structure and angiogenesis *in vitro.* *Cancer Res.* 2000; 60: 6196–200.

36. **Nemeth K, Kurucz I.** Suppression of $Ca2+$ influx by unfractionated heparin in non-excitable intact cells *via* multiple mechanisms. *Biochem Pharmacol.* 2005; 69: 929–40.

37. **Nilsson T, Zwiller J, Boynton AL, *et al.*** Heparin inhibits IP3-induced Ca^{2+} release in permeabilized pancreatic beta-cells. *FEBS Lett.* 1988; 229: 211–4.

38. **Kirn-Safran CB, D'Souza SS, Carson DD.** Heparan sulfate proteoglycans and their binding proteins in embryo implantation and placentation. *Semin Cell Dev Biol.* 2008; 19: 187–93.

39. **Bishop JR, Schuksz M, Esko JD.** Heparan sulphate proteoglycans fine-tune mammalian physiology. *Nature.* 2007; 446: 1030–7.

40. **Coombe DR, Kett WC.** Heparan sulfate-protein interactions: therapeutic potential through structure-function insights. *Cell Mol Life Sci.* 2005; 62: 410–24.

41. **Spilmann D.** Heparan sulfate: anchor for viral intruders? *Biochimie.* 2001; 83: 811–7.

42. **Mummery RS, Mulloy B, Rider CC.** The binding of human betacellulin to heparin, heparan sulfate and related polysaccharides. *Glycobiology.* 2007; 17: 1094–103.

43. **Kreuger J, Spillmann D, Li JP, *et al.*** Interactions between heparan sulfate and proteins: the concept of specificity. *J Cell Biol.* 2006; 174: 323–7.

44. **Tyagi M, Rusnati M, Presta M, *et al.*** Internalization of HIV-1 tat requires cell surface heparan sulfate proteoglycans. *J Biol Chem.* 2001; 276: 3254–61.

45. **Rabenstein DL.** Heparin and heparan sulfate: structure and function. *Nat Prod Rep.* 2002; 19: 312–31.

46. **Parish CR.** The role of heparan sulfate in inflammation. *Nature Rev Immunol.* 2006; 6: 633–43.

47. **Parish CR, Freeman C, Hulett MD.** Heparanase: a key enzyme involved in cell invasion. *Biochim Biophys Acta.* 2001; 1471: M99–108.

48. **Sanderson RD, Yang Y, Suva LJ, *et al.*** Heparan sulfate proteoglycans and heparanase-partners in osteolytic tumor growth and metastasis. *Matrix Biol.* 2004; 23: 341–52.

49. **Vlodavsky I, Ilan N, Naggi A, *et al.*** Heparanase: structure, biological functions, and inhibition by heparin-derived mimetics of heparan sulfate. *Curr Pharm Des.* 2007; 13: 2057–73.

50. **Lider O, Mekori YA, Miller T, *et al.*** Inhibition of T lymphocyte heparanase by heparin prevents T cell migration and T cell-mediated immunity. *Eur J Immunol.* 1990; 20: 493–9.

51. **Janssens JC, De Keersmaecker SC, De Vos DE, *et al.*** Small molecules for interference with cell-cell-communication systems in Gram-negative bacteria. *Curr Med Chem.* 2008; 15: 2144–56.

52. **Kuberan B, Lech MZ, Beeler DL, *et al.*** Enzymatic synthesis of antithrombinIII-binding heparan sulfate pentasaccharide. *Nat Biotechnol.* 2003; 21: 1343–6.

53. **Lamanna WC, Kalus I, Padva M, *et al.*** The heparanome–the enigma of encoding and decoding heparan sulfate sulfation. *J Biotechnol.* 2007; 129: 290–307.