Beyond Anticoagulant: Heparin as a Potential Anti-cancer Agent

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ABSTRACT

Heparin has been in the market since the last six decades due to its potential anticoagulant property. The mechanism of its anti-coagulation effect is well established. Along with its anticoagulant activity the other activity that is gathering a lot of importance now-a-days is its ability to arrest the progression of tumors specially some solid forms of tumors such as small cell lung carcinoma and pancreatic tumors. The chemically modified and light molecular weight fractions of heparin have been found to be more active in countering tumor progression. In this review we discuss a brief history of this versatile biomolecule with its clinical data and postulated mechanism of action as per recent studies. This review also highlighted the approach of heparin-conjugated nanoparticles to achieve targeted drug delivery with synergistic response in tumor cells.

INTRODUCTION

The well known anticoagulant compound heparin was discovered in the early 20th century by Howell's and his student L. Emmett Holt Jr. Howell coined the term heparin from the Greek 'hepar' meaning liver, the tissue utilized in the isolation of heparin [1]. The family of glycosaminoglycan (GAGs) consists of sulfated and non-sulfated GAGs. The sulfated class of GAGs is further subdivided into O-sulfated and N-sulfated GAGs. The heparin and long chain less sulfated polymer known as heparan sulfate (HS) comes under N-sulfated GAGs category. The presence of heparin with its similar structure has been noticed in many vertebrate and invertebrate organisms [2] including turkey [3], whale [4], camel [5], mouse [6], human [7], losbster [8], shrimp [9], mussel [10], species of clam [11] and crab [12].

The biochemical characteristic that differentiate HS to heparin is basically based on the sulfation pattern, as HS is less sulfated than heparin and possesses higher lever of acetylation on its glucosamine residue. Heparin is solely produced by the mast cells of connective tissues in the form of serglycin, a proteoglycan (PG). The complex procedure of biosynthesis of heparin in mast cells involves substantial level of sulfation with epimerization of its uronic acid. The whole process takes place in such a way that more than 80% of the glucosamine can be changed into deacetylated form with N-sulfation. The process converts 70% of the uronic acid into iduronic acid. HS on the other hand exist in association with its PG, the basic structure of HS is similar to heparin but bears lower degree of epimerization to its iduronate and also with lower level of N- and O- sulfation and charge density along the polymer [13]. The major sequence of heparin comprises of iduronate residues, often possesses sulfation at C2 positions with a N-sulfated glucosamine. Apart from sulfation at nitrogen moiety, the amino sugar part (glucosamine) is also known to have sulfation at the 6-O position. Variation in the pattern of sulfation and its distribution provides substantial heterogeneity, which can affect its biological properties [14]. The main biological property for which heparin is known all over is its ability to act as an anti-coagulant. The strong anti-coagulant property of heparin is mainly because of its ability to potentiate the anti-thrombin

(AT) based pathway for the inhibition of thrombin and factor Xa type coagulation factors.

There are numbers of affinity states between heparin and AT with its coagulation factors which ultimately terminates into high affinity interactions. There are two possible mechanisms by which heparin are known to activate AT. The first is altering the structure of AT which allows further interaction between heparin and AT results in stronger binding between these two. The conformational change also hinders the protease reactive centre loop (RCL) in AT. This alteration in structure allows interaction of RCL with factor Xa. After complex formation, the ATIII bounces back to its original low affinity binding state, which results in cleavage of RCL and liberation of heparin from the covalent complex of ATIII and factor Xa. The second mechanism of heparin anticoagulation activity is known as bridging mechanism by which a complete heparin molecule facilitate the binding of AT with thrombin. In this mechanism, a positively charge thrombin domain binds non-selectively with extended polymer of heparin. The length based interaction of heparin with serine proteases and AT has already been discussed in several studies, despite the fact that only pentasaccharide portion of heparin is required to interact with AT but a chain of at least 16 monosaccharides are required to initiate the interaction of AT with thrombin [15].

Besides of its known anticoagulant activity, heparin exhibits several other pharmacological activities due to its capacity to interact with diverse proteins. In addition, the presence of carboxylate and sulfate group endows it to possess a high negative charge (approximately -75), which allows it to have electrostatic interaction with many proteins such as growth factors, protease and chemokines [16]. Apart from its anticoagulant activity, the anti-tumor activity of heparin is gaining continuously a substantial ground in upcoming studies, due to its inhibitory effect on vascular thromboembolism and on the pathway of angiogenesis. Now-a-days researchers are focusing on generating short fractions heparin known as low-molecular-weight heparins (LMWHs) sequence which lack anticoagulant property but have more anti-metastatic effect allowing the sequence to bind with tumor growth driving proteins like P-Selectin and fibroblast growth factors [17]. This review will focus on the light molecular weight heparins and their anticancer potential.

Light molecular weight heparin as anticancer

Generally, regular fraction of heparin extracted from pork intestine or bovine lung lies between the molecular weights of 5,000 and 40,000 Daltons. Low-molecular-weight heparin is depolymerized form of unfractionated heparin comprises of heterogeneous sulfated group and are generally used to treat venous thromboembolism. The anti-tumor activity is due to its potential to inhibit tumor growth, which results improvement in survival rate [18]. There are different possible mechanisms behind this activity of heparin. One of them is the triggering the coagulation cascades or their other components like tissue factors which are instinctively involve in tumorigenesis and metastasis [19-20]. Several in-vivo studies suggests that's its antimetastatic role is probably due to its anticoagulant effect, blocking of heparanase, intervention with P-selectin-HSPG interaction and inhibitory effect on tumor adhesion and motility [21]. Dargo et al. in their study demonstrated that heparin and HS decrease cancer metastasis in the Nb rat prostatic adenocarcinoma model [22]. The new low-molecular-weight heparin (LMWH) known as revaparin not only block collaged adhesion and adenocarcinoma cells invasion of matrigel but

also decrease their intra-abdominal growth *in vivo* [23]. Many cell-based studies have demonstrated that LMWH can inhibit angiogenesis in a dose-dependent manner, while animal studies have shown that LMWH can alter tumor progression and restrict pulmonary metastasis [24-25]. The phase 2 clinical trial of dalteparin another member of LMWH has showed a promising effect in reducing ovarian cancer at a dose of 100 IU/kg [26]. In terms of clinical research, both warfarin and heparin have been tried to investigate the influence of antithrombotics on cancer. However, more detailed research has been conducted on LMWH. The first potential anticancer effect of antithrombotics was repoted in 1954 [27]. The first trial of ultra-fractionated heparin (ULH) was carried out on 277 patients, to estimate the action of ULH on the survival of small cell lung carcinoma [28]. The patients were randomized to receive chemotherapy alone or chemotherapy with dose adjusted subcutaneous UFH for 5 weeks. The group treated with UFH, attained better median survival of 317 days in comparison to 261 days of chemotherapy alone group and also with a better survival rate at 1 year (40 vs 30%), 2 year (11vs 9%), and 3 year (9 vs 6%). The preliminary data to suggesting the effect of LMWH in improving the survival of cancer patient is basically originated from an evaluation studies comparing UFH with LMWH in the first phase of thrombosis treatment [29-30]. However, the first study designed to know the effect of LMWH on survival rate in cancer was named as Fragmin Advanced Malignancy Outcome Study (FAMOUS) study in which 385 patients with lung, pancreatic, hepatic, advanced breast, ovarian, urogenital or uterine cancer were randomized to classical chemotherapy with 5000 IU daily dose of dalteparin or placebo [31]. The primary end point was mortality at one year. The estimate of 1 year survival in the dalteparin and placebo cases were 46 and 41%, respectively, for 2nd year the survival was 27 and 18% and in the 3rd year it was 21 and 12% for dalteparin and placebo group. A post hoc analysis of patients who survived more than 17 months indicated a survival benefit for dalteparin group.

The perceptible advantage of LMWH with an improved prognosis was again shown in another trial having patients with metastatic or locally advanced cancer who could not be eradicated curatively [32]. In this, 302 patients were randomized to receive their regular chemotherapy with a 6-week regime of weight adjusted nandroparin or placebo. Unlike dalteparin, here the primary end point was all-cause mortality. At the period of 6 month, the survival estimates were 61% in nandroparin group and 56% in placebo. At 12 and 24 months the relative estimates were 39 vs 27% and 21 vs 11%. In contrast to these studies, another study with a randomized 141 patients going through the advanced stage of cancer treated with LMWH or saline does not produce any significant survival advantages with LMWH [33]. This shows that LMWH cannot act as anti-tumor alone until incorporated with chemotherapy regime. Due to unsatisfactory response, the saline part was removed from the study to make it more realistic by comparing LMWH with standard care. The median survival reported for standard care was 10.5 months in comparison to 7.3 months with LMWH. Another study on 84 small cell lung carcinoma patients evaluated the efficacy of dalteperin with and without chemotherapy [34]. The study reported high overall response rate in patients, received LMWH with chemotherapy as the survival rate was 69.2% in comparison 42.5% solely by chemotherapy. A systematic study and comprehensive analysis of all these studies suggests that LMWH with chemotherapy produced better survival in cancer patients including those patients who are at advance stage of cancer [35-36].

Proposed mechanism of action

Inhibition in tumor progression due to anticoagulant property
The exact mechanism of LMWH anti-metastatic role is under scrutiny but as per the animal models and cell based research, some evidences have came into light which relates its anti-metastatic activity to its anti-coagulant activity. Clinically, the LMWH and un-fractionated heparin has been used from a long time as effective blocker of fluid phase coagulation by enhancing antithrombin inactivation of factor IIa and Xa. [37-39]. Another anti-metastatic activity of heparin is mainly by the virtue of its anticoagulant effect and is its potential to liberate tissue factor pathway inhibitor (TFPI) from vascular endothelium. The TFPI is one of the contributors in antiangiogenic activity [40-41]. Various studies have demonstrated the effect of antimetastatic activity of modified heparin with no anticoagulant activity [42-47]. These modified heparins ruled out the claim that antimetastatic activity is due to anticoagulant effect of heparin. In another study, an excellent anticoagulant agent, fondaparinux that mainly potentiate antithrombin III, had produced no effect on cancer progression at clinically accceptable dose [48-49].

Inhibition of heparinase
Another important step in the process of cancer progression, which drive the invasion of tumor cells is the debasement of various building blocks of extracellular matrix, including laminin, collagen, fibronectin and HSPGs. Cancer cells usually get this job done by employing some hydrolytic enzymes such as matrix metaloproteases, serine proteases, cysteine proteases and endoglycosidases [50-51]. Among endoglycosidases, heparinases secreted by tumor cells can sabotage various component favoring tumor invasions. The expression of heparinase is very rare in normal tissues but its expression is evident in many tumors where it significantly enhances both metastasis and angiogenesis [52] of tumors of breast [53], colon [54], ovary [55], bladder [56], pancreases [57], acute myeloid leukemia [58], non-small cell lung cancer [59] and myelomas [60]. Heparinase is a vital enzyme for the cleavage of heparin sulfate groups present in heparan sulfate proteoglycans. These HS groups possess growth factor having domain associated to the protein core with serine residue confined in ECM and in plasma membrane of cells. These domains engage growth factors, like βFGF and VEGF [61-62] and then act as their co-factor in cell signaling [63,61]. Heparanase not completely cleaves the heparan sulfate chain but it breaks the glycosidic bonds at some specific sites, generating sequences, which seems even more potent than the parent chain in activation of bound growth factors [64-65]. Various study demonstrated that heparin and some chemically altered forms of heparin blocked the tumor cell heparanase activity [66-70]. Thus, inhibiting heparinase can subsequently reduce metastatic potential of tumor [71].

Engagement with cell adhesion molecules
Invasion of tumor cells from one part of body to other part usually takes place through intravasation capability of tumor cells by which they invade blood vessels and reach to distant organs. The intravasation phase is also critical step for tumor cells from the prospect of their survival in blood vessels. To survive against the immune competent cells present in stream of blood, the tumor cells form a complex with platelets [72-73] which not only provide a cover that protect them against immune system elements but also support their anchoring on vascular endothelium [74]. This complex formation between tumor cells and platelets is mediated by interaction of glycoprotein's in the plasma membrane of tumor cells to the selectins of platelets and endothelium.

Transformed glycosylation of cell-surfaced mucins is a leading feature of tumor growth. Some of these transformations are related with carcinomas (Cancer originate from epithelial cells). *Sialyl Lewis^x* and *Lewis^a* are two of such epitopes found on the carcinoma mucins that are usually associated with tumor progression. Selectins act as adhesion receptors that recognize these altered glycosylated structures. Their physiological role in facilitating cell adhesion has already been demonstrated in inflammations, immune responses and wound repairs [75-76]. Selectins are basically adhesion molecules responsible for initiating the first step for cell adhesions and in the absence of selectins, all the steps are initiated by secondary elements of adhesion process such as integrins and other adhesion molecules are eventually delayed or do not occur. Selectins are expressed by leukocytes (L-selectin), platelets (P-selectin), and the vascular endothelium (E and P selectin). However, L selectin mainly exist on neutrophils, monocytes, and naïve lymphocytes. The secretory granules of resting platelets and endothelium stores P-selectins, which is rapidly shifted on cell surface and the process is triggered by histamine and thrombin. E-selectin is entirely produced by endothelial cells on getting activated by various inflammatory factors such as TNF-alfa, IL-I and endotoxins [77]. All these three selectins can interact with sialyted, fucosylated or with sulfated glycans on proteoglycans, glycoproteins and glycolipids. The tetrasaccharide epitope *Sialyl Lewis^x* and *Lewis^a* have been found as a minimum ligands for binding with all three types of selectin. The validation of selectins as a target for anti-cancer therapy has been already realized in animal based models. A substantial decline in platelet-tumor cell thrombi formation has resulted in diminishing of metastasis has been reported in P-selectin deficient mice [78]. Role of E-selectin in metastasis has also been validated in transgenic mice with overexpression of E-selectin, which resulted in increase in liver metastasis [79]. Reduction in metastasis is also observed in two L-selectin knocked out mice models, thus actively suggesting the role of leukocytes in aggravating metastasis [80].

Heparin has the potential to inhibit selectins even with no similarity to selectin ligands [81]. The anti-coagulation effect of heparin increase time of cancer cells to be in blood vessels by limiting their adhesion to endothelium and platelets. This makes the tumor cells more vulnerable to get neutralized by NK cells in circulation [82]. Several laboratories have analyzed the effect of heparin on selectins. In one such experiment, the UFH is injected in P and L selectin-knocked out mice shortly after the injection of melanoma cells. [83-84]. Single bolus injections of heparin have been identified to reduce metastasis in wild type mice in a similar way as in P-selectin deficient mice [83]. In addition to this, a single heparin injection in P-selectin knocked out mice produces no marked change on metastasis [74]. Interestingly, a single dose of heparin injected in L-selectin deficient mice just before to tumor cell injection resulted in further reduction in metastasis [80]. When heparin is injected shortly before or six hours after tumor cell injection, no further attenuation on metastasis in P- and L-selectin is observed. However, repeated injection of UFH causes increase in survival of mice, suggesting that heparin at such doses might have further antimetastatic activities apart from selectin blocker. A detailed characterization of non-anticoagulant heparin resulted in identification of specific heparin analogue (58% N-acetylated heparins), that holds outstanding P-selectin inhibition property while holding minimal heparinase activity and also with reduced growth factor interaction ability [85]. This heparin

analogue is found to reduce metastasis colon carcinomas and melanomas cells. Overall, these results strongly indicate that heparin block metastasis by interfering with selectin based cell-cell adhesion.

Blocking of cell surface proteoglycans
Another mechanism of heparin antimetastatic action may lies in its ability to compete with the HS group present on the Heparan sulfate proteoglycans (HSPGs) to bind with growth factors and may liberate these proteins from ECM [86]. Studies on human in vitro angiogenesis model proves that both UFH and LMWH are capable of inhibiting βFGF- induced angiogenesis by blocking the interaction βFGF with HS. In man, therapeutic dose of UFH can indeed cause a hike in plasma levels of growth factors, such as βFGF [87-89]. Contradictory to this, Soker et al. demonstrate that LMWH but not UFH can block the interaction of growth factor to their high affinity receptors due to its smaller size [90-91]. Heparin fragment of less than 18 saccharide units has been identified to reduce the activity of VEGF [92] and fragment of less than 10 saccharide units can inhibit the activity of βFGF [93-94]. In one more such contradiction to UFH, the LMWH has also been demonstrated for inhibiting βFGF and VEGF-facilitated angiogenesis *in vivo* [95]. The true relevance of HSPGs and growth factor as a potential target for heparin is very perplexed and unpredictable.

Heparin based nanoparticle and other innovative pharmaceutical approach to target tumors

The major issue today's modern chemotherapy is facing is harsh side effects of cytotoxic drugs due to their lack of specificity which can only be resolved by targeted drug delivery [94]. To overcome drug delivery issue, pharmaceutical modification in dosage form by making polymer drug conjugates are one of the approach that has been employed in past few years [90]. The approach has been shown to offer advantages in targeting tumor through a passive way by increasing the permeability and retention factor, which ultimately lead to reduction in noxious effect and increase in solubility as well as chemical stability. The advantage of polymer drug conjugates has been observed in a phase I trial where increase efficacy has been reported in forty-three patient with advanced solid tumor treated with cisplatin and poly (L-glutamic acid)-paclitaxel conjugate (PGA-PTX). Nevertheless, the challenges to find polymer with suitable physiochemical and biopharmaceutical properties are major hurdle in developing a selectively targeted polymer- drug conjugated therapy [95]. To deal with this issue, heparin, which is a biodegradable, non-cytotoxic, and water-soluble natural polysaccharide coupled with variety of paharmacological activities such anti-coagulation, anti-inflamation, anti-angiogenesis and anti-tumor [96], has allured intense attention. Taking advantage of this unique property of heparin, many heparin-drug conjugates have been developed for cancer chemotherapy [97]. In one such attempt, a LMWH based polymer drug conjugate holding two different anti-tumor drugs, Paclitaxel and all-trans-retinoic acid (ATRA) has been synthesized by Hou et al. The conjugate was reported to have less toxicity with better antitumor activity due to better permeability of nano-particles which increased the influx of paclitaxel (PTX) bound conjugate in tumor cells. The ATRA, which is known to induce regression of tumor by down regulating the survival factors of tumor and by activating of mitochondria dependent apoptosis, has played a significant role in the facilitating the transport of paclitaxel in the nucleus of tumor cell [98]. Beside heparin based nano-particles development, other approaches such as development of heparin based microcapsules loaded with anti-cancer drugs like

doxorobucin has also take pace in past few years. In one such study doxorobucin has been encapsulated with layer-by-layer assembly of heparin and chitosan. Use of chitosan is mainly to prevent degradation of heparin from heparanase and also to facilitate the cellular uptake of heparin as the heparin is negatively charge that prevents it to move across the cell membrane, layer of positively charge chitosan applied to counter this effect. Thus, a synergistic response has been obtained against A549 cell lines [99]. Beside conjugation of two active anti-tumor agents, the attempt to make heparin-based nanoparticles also employed conjugation of florescent dye labeled heparin with gold nanoparticles.

Gold nanoparticles are widely used in many studies for their role in detection of biomolecules [100], selective marking of cells and proteins [101], killing tumor cells by hyper-thermal treatment and delivering therapeutic agent inside cells. In a study by Lee et al., heparin immobilized gold nanoparticles were prepared to optically identify the metastatic stage of tumor cells as well as to initiate apoptosis-induced tumor death by loading heparin into the cells. The nanoparticles were prepared by sealing the florescent heparin at the surface of gold nano-particles via gold-thiol interaction to obtain florescence resonance energy transfer (FRET). These nanoparticles were further conjugated with arginine-glycine-aspartic acid, a cell adhesive peptide to make it more effective for inducing apoptosis. The study achieved acute apoptosis in $\alpha_v\beta_3$ integrin expressing cancer cells due to targeted influx of heparin in cytoplasm of cancer cells [102]. In addition to all this innovations, Alam et al. recently conjugated a potent anti-angiogenic analogue of heparin (LHT7) with tetrameric deoxycholic acid to prepare an oral formulation of heparin with better bioavailability [103]. The LHT7 has also been studied for its conjugation with reduced graphene oxide nanoparticles in a separate study. In this, light molecular weight heparin analogue coated with nano sheet of reduced graphene oxide is further loaded with doxorubin. The particles are found to have a better dispersion in tumor vasculature of KB carcinoma bearing mice with better efficacy possibly due to synergistic effect of doxorubicin and LHT7 [104].

Conclusion
Heparin is the most negatively charged and important member of the GAG family and is commercially available in the market as a potential anticoagulant. Presence of heparin in various species has already been well documented but major commercial sources are still limited to porcine intestine and bovine lungs. Biological properties of heparin have been found to largely depend on its sulfation pattern. The properties also differ on the basis of its distribution in various vertebrates and invertebrates. The anticoagulant features of heparin have already been well studied and documented in various studies. The mechanism of anticoagulant activity of heparin is well established and known. Beside from its anticoagulant role, heparin has been explored in the past few decades for its anti-tumor properties, a role which gained much momentum after the development of some light molecular weight fractions of heparins (LMWH) along with unfractionated heparin (UFH) and non sulfated heparins with little or almost no anticoagulant properties. The process of tumor growth has been known to be highly complicated as many hallmarks of cancer such as angiogenesis and metastasis use different tactics to make the progression simplified for tumor cells. There is enough evidence suggesting heparin as a potential therapeutic agent against cancer. Various experimental studies have already demonstrated the efficacy of heparin in arresting tumor growth both in vitro and in vivo models. Clinical trials of some of the

LMWH have already shown better survival rate and regression of solid tumors especially in lung carcinoma patients. The clarity on its actual mechanism of action is still not clear. As some studies suggest that it interfere the progression of tumor by its anticoagulant property while other point out its interaction potential to engage cell adhesion molecules and proteoglycans. A synergistic and selective approach of heparin-based nanoparticles loaded with classical anti-cancer drugs such as paclitaxel and doxorubicin to target tumor cells, has been obtained by several studies. It can be concluded that heparin have a potential to serve itself as an anticancer agent or can complement the treatment in combination with other anti-cancer agent to minimize the harsh side effects and chemotherapy. Apart from the need to give much emphasis on a clear cut mechanism of mechanism of its action there are some other issue which are also needed to be addressed such as: what type of heparin, what dose is needed, what duration of administration are optimum for better anticancer outcome. These questions urgently needed to be answered so that design of prospective randomized clinical trial especially in patients with early metastasis can take place.

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References

[1] Wardrop D, Keeling D. The story of the discovery of heparin and warfarin. Br J Haem. 2008; 141(6):757–763.

[2] Nader HB, Chavante SF, Oliveira FW, Medeiros GF, Leite EL, Toma L, Tersariol ILS, Porcionatto MA, Dietrich CP. Heparan sulfates and heparins: similar compounds performing the same functions in vertebrates and invertebrates? Braz J Med Biol Res. 1999; 32:529-538.

[3] Warda M, Mao W, Toida T, Linhardt RJ. Turkey intestine as a commercial source of heparin? Comparative structural studies of intestinal avian and mammalian glycosaminoglycans. Comp Biochem Physiol B Biochem Mol Biol. 2003; 134(1):189–97.

[4] Ototani N, Kikuchi M, Yosizawa Z. Comparative studies on the structures of highly active and relatively inactive forms of whale heparin. J Biochem. 1981; 90:241–246.

[5] Warda M, Gouda E M, Toida T, Chi L, Linhardt RJ. Isolation and characterization of raw heparin from dromedary intestine: evaluation of a new source of pharmaceutical heparin. Comp Biochem Physiol C Toxicol Pharmacol. 2003; 136:357– 365.

[6] Bland CE, Ginsburg H, Silbert JE, Metcalfe DD. Mouse heparin proteoglycan Synthesis by mast cell fibroblast monolayers during lymphocyte dependent mast cell proliferation. J Biol Chem. 1982; 257:8661–8666.

[7] Linhardt RJ, Ampofo SA, Fareed J, Hoppensteadt D, Folkman J, Mulliken JB. Isolation and characterization of human heparin. Biochemistry. 1992; 31: 12441–12445.

[8] Hovingh P, Linker A. An unusual heparan sulfate isolated from lobsters (*Homarus americanus*). J Biol Chem. 1982; 257:9840–9844.

[9] Dietrich CP, Paiva JF, Castro RAB, Chavante SF, Jeske W, Fareed J, Gorin, PA, Nadher HB. Structural features and anticoagulant activities of a novel natural low-molecular-weight heparin from the shrimp Penaeus brasiliensis. Biochim Biophys Acta. 1999; 1428: 273–283.

[10] Hovingh P, Linker A. Glycosaminoglycans in *Anodonta californiensis*, a freshwater mussel. Biol Bull. 1993;185:263–276.

[11] Pejler G, Danielsson A, Bjork I, Lindahl U, Nader HB, Dietrich CP. Structure and antithrombin-binding properties of heparin isolated from the clams *Anomalocardia brasiliana* and *Tivela mactroides*. J Biol Chem. 1987; 262:11413–11421.

[12] Medeiros GF, Mendes A, Castro RAB, Baffl EC, Nader HB, Dietrich CP. Distribution of sulfated glycosaminoglycans in the animal kingdom: widespread occurrence of heparin like compounds in invertebrates. Biochim Biophys Acta. 2000; 1475:287– 294.

[13] Azmi NS, Fernig DG. Heparan sulfate surfaces to probe the functions of the master regulator of the extracellular spaces. Handbook of Biofunctional Surfaces. Pan Standford Publishing. Wolfgang Knoll. 16: 2013; 591-609.

[14] Chavante SF, Brito, AS, Lima M, Yates E, Nader H, Guerrini M, Torri G, Bisio A. A heparin-like glycosaminoglycan from shrimp containing high levels of 3-O-sulfated D-glucosamine groups in an unusual trisaccharide sequence. Carbohydr Res. 2014; 390: 59–66.

[15] Petitou M, Duchaussoy P, Driguez PA, Jaurand G, Herault JP, Lormeau JC, Boeckel CAV, Herbert JM. First synthetic carbohydrates with the full antocoagulant properties of heparin. Angew Chem Int Ed Engl. 1998; 37: 3009–3014.

[16] Liang Y, Kiick KL. Heparin-functionalized polymeric biomaterials in tissue engineering and drug delivery applications. Acta Biomaterialia. 2014; 10(4):1588–1600.

[17] Li Y, Luo JY, Cui HF, Zheng L, Du SS. Study on anti-metastasis of heparin derivatives as ligand antagonist of P-selectin. Biomed Pharmacother. 2010; 64(10): 654–658.

[18] Zacharski LR, Lee AY. Heparin as an anticancer therapeutic. Expert Opin Investig Drugs. 2008; 17(7):1029-1037.

[19] Nash GF, Walsh DC, Kakkar AK. The role of the coagulation system in tumour angiogenesis. Lancet Oncol. 2001; 2:608-613.

[20] Ruf W, Disse J, Carneiro-Lobo TC, Yokota N, Schaffner F. Tissue factor and cell signalling in cancer progression and thrombosis. J Thromb Haemost. 2011; 9(1): 306-15.

[21] Yip GW, Smollich M, Gotte M. Therapeutic value of glycosaminoglycans in cancer. Mol Cancer Ther. 2006; 5:2139–2148.

[22] Drago JR, Weed P, Fralisch A. The evaluation of heparin in control of metastasis of Nb rat androgen-insensitive prostate carcinoma. Anticancer Res. 1984; 4:171–182.

[23] Pross M, Lippert H, Misselwitz F, Nestler G, Kruger S, Langer H, Halangk W, Schulz HU. Low-molecular-weight heparin (reviparin) diminishes tumor cell adhesion and invasion in vitro, and decreases intraperitoneal growth of colonadeno-carcinoma cells in rats after laparoscopy. Thromb Res. 2003; 110(4), 215–220.

[24] Amirkhosravi A, Mousa S A, Amaya M, Francis J L. Anti-metastatic effect of tinzaparin, a low-molecular-weight heparin. Journal Thromb Haemost. 2003; 1(9):1972-1976.

[25] Sciumbata T, Caretto P, Pirovano P, Pozzi P, Cremonesi P, Galimberti G, Leoni F, Marcucci F. Treatment with modified heparins inhibits experimental metastasis formation and leads, in some animals, to long-term survival. Invas Metas. 1996; 16(3):132-143.

[26] Elit LM, Lee AY, Parpia S, Swystun LL, Liaw PC, Hoskins P, Julian DH, Julian JA, Levine MN. Dalteparin low-molecular-weight heparin (LMWH) in ovarian cancer: a phase II randomized study. Thromb Res. 2012; 130(6): 894–900.

[27] Albert-Weil J, Nehorias J. Two cases of neoplasms not justified for classical therapy, treated with intravenous injections of heparin and intramuscular injections of leech extracts. Pathol Gen Physiol Clin. 1954; 54:1014-1020.

[28] Lebeau B, Chastang C, Brechot JM, Capron F, Dautzenberg B, Delaisements C, Mormet M, Brun J, Hurdebourcq JP, Lemarie E. Subcutaneous heparin treatment increases survival in small cell lung cancer. "Petites Cellules" Group. Cancer. 1994; 74:38-45.

[29] Green D, Hull RD, Brant R, Pineo GF. Lower mortality in cancer patients treated with low-molecular-weight versus standard heparin. Lancet. 1992; 339:1476.

[30] Gould MK, Dembitzer AD, Doyle RL, Hastie TJ, Garber AM. Low-molecular-weight heparins compared with unfractionated heparin for treatment of acute deep venous thrombosis. A meta-analysis of randomized, controlled trials. Ann Intern Med. 1999; 130:800-809.

[31] Kakkar AK, Levine MN, Kadziola Z, Lemoine NR, Low V, Patel HK, Rustin G, Thomas M, Quigley M, Williamson RC. Low-molecular-weight heparin, therapy with dalteparin, and survival in advanced cancer: the fragmin advanced malignancy outcome study (FAMOUS). J Clin Oncol. 2004; 22:1944-8.

[32] Klerk CP, Smorenburg SM, Otten HM, Lensing AW, Prins MH, Piovella F, Prandoni P, Boss MM, Richel DJ, Tienhoven G, Buller HR. The effect of low-molecular-weight heparin on survival in patients with advanced malignancy. J Clin Oncol. 2005; 23:2130-2135.

[33] Sideras K, Schaefer PL, Okuno SH, Sloan JA, Kutteh L, Fitch TR, Dakhil SR, levitt R, Alberts SR, Morton RF, Rowland KM, Novotny PJ, Loprinzi CL. Low-molecular-weight heparin in patients with advanced cancer: a phase 3 clinical trial. Mayo Clin Proc. 2006; 81:758-767.

[34] Altinbas M, Coskun HS, Er O, Ozkan M, Eser B, Unal A, Cetin M, Soyuer S. A randomized clinical trial of combination chemotherapy with and without low- molecular-weight heparin in small cell lung cancer. J Thromb Haemost. 2004; 2:1266-1271.

[35] Lazo-Langner A, Goss GD, Spaans JN, Rodger MA. The effect of low- molecular-weight heparin on cancer survival. A systematic review and meta-analysis of randomized trials. J Thromb Haemost. 2007; 5:729-737.

[36] Noble S. Heparins and cancer survival: where do we stand? Thromb Res. 2014; 133 (2):133–138.

[37] Borsig L, Wong R, Feramisco J, Nadeau DR, Varki NM, Varki A. Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis. Proc Natl Acad Sci U S A. 2001; 98:3352–3357.

[38] Varki NM, Varki A. Heparin Inhibition of Selectin-Mediated Interactions during the Hematogenous Phase of Carcinoma Metastasis: Rationale for Clinical Studies in Humans. Semin Thromb Hemost. 2002; 28:53–66.

[39] Im JH, Fu W, Wang H, Bhatia SK, Hammer DA, Kowalska MA, Muschel RJ. Coagulation facilitates tumor cell spreading in the pulmonary vasculature during early metastatic colony formation. Cancer Res. 2004; 64:8613–8619

[40] Vlodavsky I, Ilan N, Nadir Y, Brenner B, Katz BZ, Naggi A, Torri G, Casu B, Sasisekharan R. Heparanase, heparin and the coagulation system in cancer progression. Thromb Res. 2007;120 Suppl 2:112–120.

[41] Mousa SA, Petersen LJ. Anti-cancer properties of low-molecular-weight heparin: preclinical evidence. Thromb Haemost. 2009; 102:258–267.

[42] Hostettler N, Naggi A, Torri G, Ishai-Michaaeli R, Casu B, Vlodoavsky l, Borsig L. P-selectin- and heparanase-dependent antimetastatic activity of non-anticoagulant heparins. FASEB J. 2007; 21:3562–3572.

[43] Kragh M, Binderup L, Vig Hjarnaa PJ, Bramm E, Johansen KB, Frimundt Peterson C. Non-anti-coagulant heparin inhibits metastasis but not primary tumor growth. Oncol Rep. 2005;14:99–104.

[44] Yoshitomi Y, Nakanishi H, Kusano Y, Munesue S, Tatematsu M, Yamashina I, Okayama M. Inhibition of experimental lung metastases of Lewis lung carcinoma cells by chemically modified heparin with reduced anticoagulant activity. Cancer Lett. 2004; 207:165–174.

[45] Lapierre F, Holme K, Lam L, Tressier RJ, Storm N, Wee J, Stack RJ, Castellot J, Tyrell DJ. Chemical modifications of heparin that diminish its anticoagulant but preserve its heparanase-inhibitory, angiostatic, anti-tumor and anti- metastatic properties. Glycobiology.1996; 6:355–366.

[46] Borsig L, Wong R, Hynes RO, Varki NM, Varki A. Synergistic effects of L- and P-selectin in facilitating tumor metastasis can involve non-mucin ligands and implicate leukocytes as enhancers of metastasis. Proc Natl Acad Sci USA. 2002; 99:2193–2198.

[47] Vlodavsky I, Eldor A, Haimovitz-Friedman A, Matzner Y, Ishai-Michaeli R, Lider O, Naparstek Y, Cohen IR, Fuks Z. Expression of heparanase by platelets and circulating cells of the immune system: possible involvement in diapedesis and extravasation. Invas Meta. 1992; 12:112–127.

[48] Stevenson JL, Choi SH, Varki A. Differential metastasis inhibition by clinically relevant levels of heparins–correlation with selectin inhibition, not antithrombotic activity. Clin Cancer Res. 2005;11: 7003–7011.

[49] Ludwig RJ, Alban S, Bistrian R, Boehncke WH, Kaufimann R, Hensschler R, Gille J. The ability of different forms of heparins to suppress P-selectin function in vitro correlates to their inhibitory capacity on blood borne metastasis in vivo. Thromb Haemost. 2006; 95:535–540.

[50] Nakajima M, Irimura T, Nicolson GL. Heparanases and tumor metastasis. J Cell Biochem. 1988; 36:157–167.

[51] Vlodavsky I, Korner G, Ishai-Michaeli R, Bashkin P, Bar-Shavit R, Fuks Z. Extracellular matrix- resident growth factors and enzymes: possible involvement in tumor metastasis and angiogenesis. Cancer Meta. 1990; 9:203–226.

[52] Vlodavsky I, Goldshmidt O, Zcharia E, Atzmon R, Rangini-Guatta Z, Elkin M, Peretz T, Friedmann Y. Mammalian heparanase: involvement in cancer metastasis, angiogenesis and normal development. Semin Cancer Biol. 2002; 12:121–129.

[53] Maxhimer JB, Quiros RM, Stewart R, Dowalatshahi K, Gattuso P, Fan M, Prinz RA, Xu X Heparanase-1 expression is associated with the metastatic potential of breast cancer. Surgery 2002; 132:326–333.

[54] Friedmann Y, Vlodavsky I, Aingorn H, AvivA, Peretz T, Pecker l, Pappo O. Expression of heparanase in normal, dysplastic, and neoplastic human colonic mucosa and stroma. Evidence for its role in colonic tumorigenesis. Am J Pathol. 2000; 157:1167–1175.

[55] Ginath S, Menczer J, Friedmann Y, Aingorm H, Aviva A, Tajimak, Dantes A, Glezerman M, Vlodavasky I, Amsterdam A. Expression of heparanase, Mdm2, and erbB2 in ovarian cancer. Int J Oncol. 2001; 18:1133–44.

[56] Gohji K, Hirano H, Okamoto M, Kitazawa S. Toyoshima M, Dong J, Katsuoko Y, Nakajima M. Expression of three extracellular matrix degradative enzymes in bladder cancer. Int J Cancer 2001; 95:295–301.

[57] Koliopanos A, Friess H, Kleeff J, Shi X, Liao Q, Pecker I, Vlodovasky I, Zimmermann A, Buchler MW. Heparanase expression in primary and metastatic pancreatic cancer. Cancer Res 2001; 61:4655–4659.

[58] Bitan M, Polliack A, Zecchina G, et al. Heparanase expression in human leukemias is restricted to acute myeloid leukemias. Exp Hematol. 2002; 30:34–41.

[59] Takahashi H, Ebihara S, Okazaki T, Suzuki S, Asada M, Kubo H, Sasaki H. Clinical significance of heparanase activity in primary resected non-small cell lung cancer. Lung Cancer. 2004; 45:207–214.

[60] Yang Y, Macleod V, Bendre M, Huang Y, Thesus AM, MiaoHQ, Kussie P, Yaccoby S, Suva LJ, Kelly T Sanderson RD. Heparanase promotes the spontaneous metastasis of myeloma cells to bone. Blood. 2005; 105:1303–1309.

[61] Ishai-Michaeli R, Eldor A, Vlodavsky I. Heparanase activity expressed by platelets, neutrophils, and lymphoma cells releases active fibroblast growth factor from extracellular matrix. Cell Regul. 1990; 1:833–842.

[62] Whitelock JM, Murdoch AD, IozzoRV, Underwood PA.The degradation of human endothelial cell-derived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases. J Biol Chem. 1996; 271:10079–10086.

[63] Sanderson RD, Yang Y, Suva LJ, Kelly T. Heparan sulfate proteoglycans and heparanase—partners in osteolytic tumor growth and metastasis. Matrix Biol. 2004; 23:341–52.

[64] Elkin M, Ilan N, Ishai-Michaeli R, Friedman Y, Papo O, Pecker I, Vlodavsky I. Heparanase as mediator of angiogenesis: mode of action. FASEB J. 2001; 15:1661–1663.

[65] Kato M, Wang H, Kainulainen V, Fitzgerals ML, Ledbetter S, Ornitz DM, Bernfield M. Physiological degradation converts the soluble syndecan-1 ectodomain from an inhibitor to a potent activator of FGF-2. Nat Med. 1998; 4:691–697.

[66] Irimura T, Nakajima M, Nicolson GL. Chemically modified heparins as inhibitors of heparan sulfate specific endo-beta-glucuronidase (heparanase) of metastatic melanoma cells. Biochemistry. 1986; 25:5322–5328.

[67] Vlodavsky I, Mohsen M, Lider O, Svahn CM, Ekre HP, Vigoda M, Ishai-Michaeli R, Peretz T. Inhibition of tumor metastasis by heparanase inhibiting species of heparin. Invas Meta. 1994; 14:290–302.

[68] Parish CR, Coombe DR, Jakobsen KB, Bennett FA, Underwood PA. Evidence that sulphated polysaccharides inhibit tumour metastasis by blocking tumour-cell-derived heparanases. Int J Cancer. 1987;40:511–518.

[69] Vlodavsky I, Friedmann Y, Elkin M, Aingorn H, Atzmon R, Ishai-Michaeli R, Bitan M, Pappo O, Peretz T, Michal I, Spector L, Pecker I. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nat Med. 1999; 5:793–802.

[70] Poggi A, Rossi C, Casella N, Bruno C, Sturiale L, Dossi C, Naggi A. Inhibition of B16-BL6 melanoma lung colonies by semisynthetic sulfaminoheparosan sulfates from E.coli K5 polysaccharide. Semin Thromb Hemost. 2002; 28:383–392.

[71] Miao HQ, Elkin M, Aingorn E, Ishai-Michaeli R, Stein CA, Vlodavsky L. Inhibition of heparanase activity and tumor metastasis by laminarin sulfate and synthetic phosphorothioate oligodeoxynucleotides. Int J Cancer. 1999; 83:424–431.

[72] Gasic GJ. Role of plasma, platelets, and endothelial cells in tumor metastasis. Cancer Meta. 1984; 3:99–114.

[73] Nieswandt B, Hafner M, Echtenacher B, Mannel DN. Lysis of tumor cells by natural killer cells in mice is impeded by platelets. Cancer Res.1999; 59:1295–1300.

[74] Borsig L, Wong R, Feramisco J, Nadeau DR, Varki NM, Varki A. Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis. Proc Natl Acad Sci USA. 2001; 98:3352–3357.

[75] Subramaniam M, Saffaripour S, Van De WL, Frenette PS, Mayadas TN, Hynes RO. Role of endothelial selectins in wound repair. Am J Pathol. 1997; 150:1701–1709.

[76] Talbott GA, Sharar SR, Harlan JM, Winn RK. Leukocyte-endothelial interactions and organ injury: the role of adhesion molecules. New Horiz. 1994; 2:545–554.

[77] Cummings RD, Smith DF. The selectin family of carbohydrate-binding proteins: structure and importance of carbohydrate ligands for cell adhesion. Bioessays. 1992; 14:849–856.

[78] Lee AE, Rogers LA, Longcroft JM, Jeffery RE. Reduction of metastasis in a murine mammary tumour model by heparin and polyinosinic-polycytidylic acid. Clin Exp Metastasis. 1990; 8:165–171.

[79] Biancone L, Araki M, Araki K, Vassalli P, Stamenkovic I. Redirection of tumor metastasis by expression of E-selectin in vivo. J Exp Med. 1996; 183:581–587.

[80] Borsig L, Wong R, Hynes RO, Varki NM, Varki A. Synergistic effects of L- and P-selectin in facilitating tumor metastasis can involve non-mucin ligands and implicate leukocytes as enhancers of metastasis. Proc Natl Acad Sci USA. 2002; 99:2193–2198.

[81] Koenig A, Norgard-Sumnicht K, Linhardt R, Varki A. Differential interactions of heparin and heparan sulfate glycosaminoglycans with the selectins. Implications for the use of unfractionated and low-molecular-weight heparins as therapeutic agents. J Clin Invest. 1998; 101:877–889.

[82] Borsig L, Wong R, Feramisco J, Nadeau DR, Varki NM, Varki A. Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis. Proc Natl Acad Sci USA. 2001; 98:3352–3357.

[83] Borsig L, Wong R, Hynes RO, Varki NM, Varki A. Synergistic effects of L- and P-selectin in facilitating tumor metastasis can involve non-mucin ligands and implicate leukocytes as enhancers of metastasis. Proc Natl Acad Sci USA. 2002; 99:2193–2198.

[84] Ludwig RJ, Boehme B, Podda M, Henschler R, Jager E, Tandi C. Endothelial P-selectin as a target of heparin action in experimental melanoma lung metastasis. Cancer Res. 2004;64:2743–5270.

[85] Stevenson JL, Varki A, Borsig L. Heparin attenuates metastasis mainly due to inhibition of P- and L-selectin, but non-anticoagulant heparins can have additional effects. Thromb Res. 2007; 120 (2):107–111.

[86] Colin S, Jeanny JC, Mascarelli F, Al-Mahmood S, Courtois Y, Labarre J. In vivo involvement of heparan sulfate proteoglycan in the bioavailability, internalization, and catabolism of exogenous basic fibroblast growth factor. Mol Pharmacol. 1999; 55:74–82.

[87] Collen A, Smorenburg SM, Peters E, Lupu F, Koolwijk P, Noorden VC. Unfractionated and low-molecular-weight heparin affect fibrin structure and angiogenesis in vitro. Cancer Res. 2000; 60:6196–6200.

[88] D'Amore PA. Capillary growth: a two-cell system. Semin Cancer Biol. 1992; 3:49–56.

[89] Folkman J, Weisz PB, Joullie MM, Li WW, Ewing WR. Control of angiogenesis with synthetic heparin substitutes. Science. 1989; 243:1490–1493.

[90] Soker S, Goldstaub D, Svahn CM, Vlodawsky l, Levi BZ, Neufeld G. Variations in the size and sulfation of heparin modulate the effect of heparin on the binding of VEGF165 to its receptors. Biochem Biophys Res Commun. 1994; 203:1339–1347.

[91] Gohji K, Hirano H, Okamoto M, Kitazawa S, Toyoshima M, Katauoko Y. Expression of three extracellular matrix degradative enzymes in bladder cancer. Int J Cancer. 2001; 95:295–301.

[92] Lepri A, Benelli U, Bernardini N, Bianchi F, Lupetti M, Danesi R. Effect of low molecular weight heparan sulphate on angiogenesis in the rat cornea after chemical cauterization. J Ocul Pharmacol. 1994; 10:273–280.

[93] Jayson GC, Gallagher JT. Heparin oligosaccharides: inhibitors of the biological activity of bFGF on Caco-2 cells. Br J Cancer. 1997; 75:9–16.

[94] Wagner AD, Grothe W, Haerting J, Kleber G, Grothey A, Fleig WE. Chemotherapy in advanced gastric cancer: a systematic review and meta-analysis based on aggregate data. J Clin Oncol. 2006; 24:2903-2909.

[95] Nieves DJ, Azmi NS, Xu R, Levy R, Yates E, Fernig DG. Monovalent maleimide functionalization of gold nano particles via copper-free click chemistry. Chem Comm. 2014; 50: 13157-13160.

[96] Dosio F, Brusa P, Crosasso P, Arpicco S, Cattel L. Preparation, characterization and properties in vitro and in vivo of a paclitaxel albumin conjugate. J Control Rel. 1997;47:293-304.

[97] Singla AK, Garg A, Aggarwal D. Paclitaxel and its formulations. Int J Pharm. 2002; 235:179-192.

[98] Hou L, Yao J, Zhou J, Zhiang Q. Biomaterials Pharmacokinetics of a paclitaxel-loaded low-molecular-weight heparin-all-trans-retinoid acid conjugate ternary nanoparticulate drug delivery system. Biomaterials. 2012; 33(21): 5431–5440.

[99] Chen JX, Liang Y, Liu W, Huang J, Chen H J. Fabrication of doxorubicin and heparin co-loaded microcapsules for synergistic cancer therapy. Int J Biol Macromol. 2014; 69: 554–560.

[100] Zhao W, Lam JCF, Chiuman W, Brook MA, Li Y. Enzymatic cleavage of nucleic acids on gold nanoparticles: a generic platform for facile colorimetric biosensors. Small. 2008; 4:810-816.

[101] Lin CJ, Yang T, Lee C, Huang SH, Sperling RA, Zanella M. Synthesis, characterization, and bioconjugation of fluorescent gold nanoclusters toward biological labeling applications. ACS Nano. 2009; 3:395-401.

[102] Lee K, Lee H, Bae KH, Park TG. Heparin immobilized gold nanoparticles for targeted detection and apoptotic death of metastatic cancer cells. Biomaterials. 2010; 31(25):6530–6536.

[103] Lee DY, Kim SK, Kim YS, Son DH, Nam JH, Kim IS. Suppression of angiogenesis and tumor growth by orally active deoxycholic acid-heparin conjugate. J Control Rel.2007; 118(3):310–317.

[104] Shim G, Kim JY, Han J, Chuang SW, Lee S, Byun Y. Reduced graphene oxide nanosheets coated with an anti-angiogenic anticancer low-molecular-weight heparin derivative for delivery of anticancer drugs. J Control Rel. 2014; 189:80–89.