Reviews • POST SCREEN

LMWH and its derivatives represent new rational for cancer therapy: construction strategies and combination therapy

Shi Du, Yao Yu, Cheng Xu, Hui Xiong, Shan Yang and Jing Yao

State Key Laboratory of Natural Medicines and Jiangsu Key Laboratory of Drug Stability of Biopharmaceuticals, Department of Pharmaceutics, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing, 210009, China

Low-molecular-weight heparin (LMWH) has attracted increasing attention as a tumor treatment because of its board range of physiological functions. Over the past decade, diverse LMWH derivatives have increased the variety of antitumor strategies available, serving not only as anti-tumor agents, but also as drug delivery platforms. In this review, we introduce the basic strategy for structural modification of LMWH to attenuate its antitumor activity while reducing its risk of bleeding and immune responses, as well as highlighting current applications of LMWH and its derivatives in cancer therapy. We select representative drug delivery systems involving LMWH derivatives and discuss the construction principles and therapeutic effects associated with their use. We also analyze progress made in the development of antitumor combination therapies, in which LMWH has shown synergistic or combined effects with other treatment strategies.

Introduction

In addition to cancer-related venous thromboembolism (VTE), it is now widely accepted that Low-molecular-weight heparin (LMWH) can directly affect the tumor biology by impacting cancer cell adhesion, proliferation, metastasis, and angiogenesis [1]. The interference of LMWH with biological processes in tumors occurs through its interactions with various mediators, such as growth factors, adhesion molecules, and enzymes (Fig. 1). Thus, LMWH could inhibit many steps in the biological activities of cancer cells, ultimately triggering a cascade of biochemical responses, influencing tumor progression, growth, or invasion. LMWH also shows potential to enhance the effects of anticancer therapeutics, as a nanotechnology and combination anticancer therapy. Here, we summarize the rapidly growing applications of LMWH and its derivatives in nanomedicine, especially as combined antitumor treatments.

Strategies to develop LMWH-based formulations

LMWH gained popularity over unfractionated heparin (UFH) for its wider range of applications, such as long-term prophylaxis of VTE. Although similar to UFH, LMWH can cause an immune response called heparin-induced type II thrombocytopenia, which involves the production of antibodies against the heparin-platelet factor 4 (PF4) complex [2]. In addition, nonspecific binding of long-chain heparin to coagulation factors, such as von Willebrand factor (vWF), also increases the bleeding risks. Thus, bleeding avoidance and promotion of anticancer effects are a priority for LMWH modification. At the pharmaceutical formulation level, another disadvantage of heparin-based treatment is that its large molecular weight and charge necessitate parenteral administration, which compromises patient compliance and drug efficiency. The emergence of LMWH-based nano drug delivery systems enables reliable *in vivo* therapeutic outcomes. Therefore, developing nano-systems through the structural modification of LMWH has emerged as an important drug development strategy.

Enhancing the sulfation of LMWH

Enhanced binding affinity for, and inhibition of, angiogenesis-related proteins could be crucial for potentiating the antiangiogenic activity of LMWH. Given that heparin-binding domains (HBD) are characterized by clusters of basic amino acids with positive charges, endowing LMWH with an additional negative charge might further enhance the LMWH–HBD binding affinity.

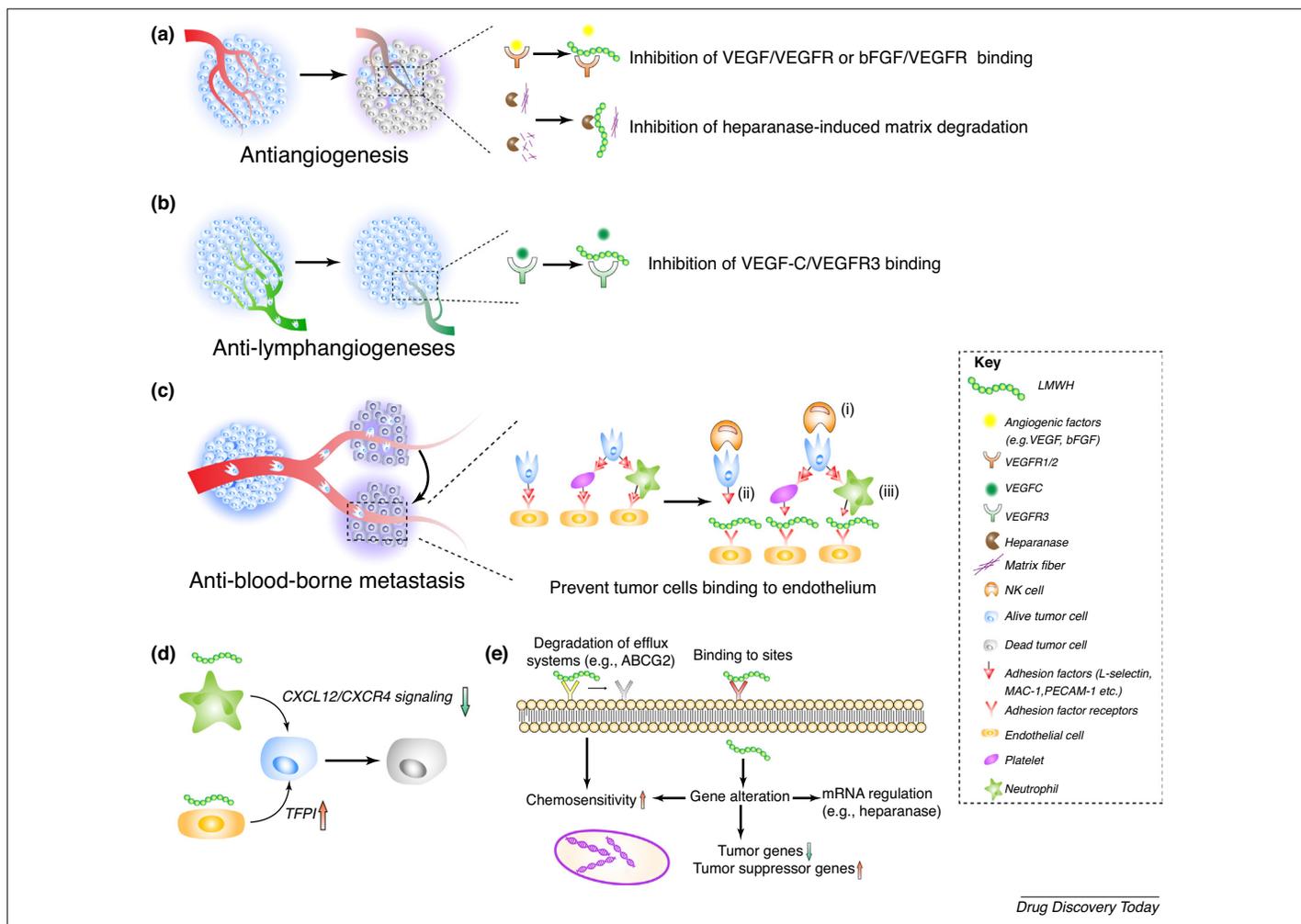*Corresponding author:* Yao, J. (yaoj3@163.com)

FIGURE 1

Mechanisms for LMWH-based antitumor therapy. **(a)** antiangiogenesis; **(b)** antilymphangiogenesis; **(c)** antimetastasis: (i) inhibiting adhesion of tumor cells to endothelium; (ii) inhibiting tumor cell–platelet–neutrophil complex formation; and (iii) promoting tumor cell clearance by natural killer (NK) cells; **(d)** chemotaxis and endothelium involved in tumor inhibition; and **(e)** direct regulation of tumor cell function. Abbreviation: VEGF, vascular endothelial growth factor. bFGF, basic fibroblast growth factor.

For example, LMWH has been conjugated with hydrophilic poly-sulfonated suramin that also has a binding affinity for HBD because of charge-based interactions. Computer simulations and surface plasmon resonance (SPR) studies demonstrated that the stronger antiangiogenic effect and anticoagulant activity of LMWH could be because binding of the suramin fragment to LMWH (resulting in LHsura) increases its binding affinity for vascular endothelial growth factor (VEGF)$_{165}$. In addition, such modification enhanced the antiangiogenetic effects of LHsura as evaluated in several VEGF$_{165}$-induced angiogenesis studies and resulted in tumor growth inhibition in a murine model of squamous cell carcinoma (SCC7) [3]. Therefore, increasing the amount of net negative charge by increasing the number of sulfate groups could be a strategy to improve the binding affinity of LMWH to VEGF (Fig. 2ai).

However, the additional sulfate groups might compete with the pre-existing sulfate groups of LMWH against the interaction with the cluster of Arg residues of the general heparin-binding site of the HBD. LMWH-sodium taurocholate conjugates (LHT7), which have a sulfate group at the terminal moieties of sodium

taurocholate, show stronger binding of LHT7 to VEGF compared with taurine directly saturated LMWH. This is because sodium taurocholate binds to LMWH and exposes new binding sites (Arg 112 and Arg 165) on the HBD, which are farther away from the general heparin-binding sites of HBD, thus avoiding competition with any pre-existing sulfate groups. The sterane core of the sodium taurocholate complex could deliver sulfate groups to more distant targets, resulting in significant improvements in VEGF binding capacity [4].

Discovery of new binding sites

Nonelectrostatic interactions, such as hydrophobic interactions, also result in additional LMWH–protein binding affinity, which explains the enhanced antiangiogenic activities of many LMWH hydrophobic derivatives [5,6]. Although the occupation of some carboxylic acid groups of LMWH decreases its negative charge, the remaining carboxylic acid groups and sulfate groups can interact with basic residues of proteins, whereas hydrophobic modifications can induce hydrophobic interactions with the hydrophobic domains of proteins (Fig. 2aii). In addition, conjugation of bulky,
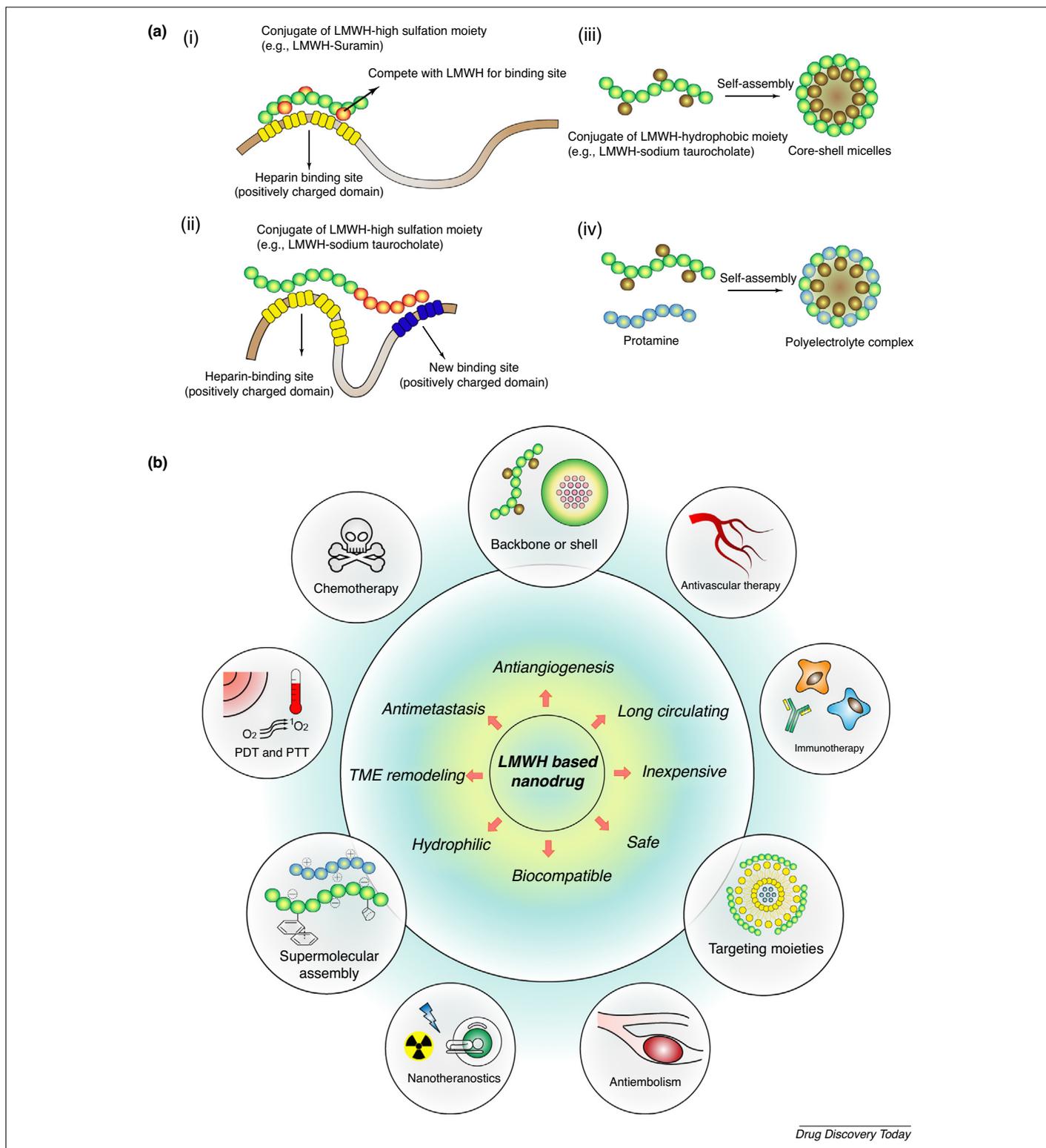
FIGURE 2

Construction of low-molecular-weight (LMWH) derivatives and LMWH-based combination therapy. **(a)** LMWH covalent modifications: (i) increase sulfation; (ii) discover new binding sites; (iii) hydrophobic groups cause severe conformational changes of angiogenic factors; (iv) conjugate assembly via hydrophobic groups and electrostatic interactions to neutralize the anticoagulation effects of heparin and stabilize the nanocomplex. **(b)** LMWH-based nanoplatform and combination therapy with other antitumor treatments. Abbreviations: PDT, photodynamic therapy; PTT, photothermal therapy.

rigid hydrophobic moieties (e.g., deoxycholic acid) cause structural changes, which decrease the flexibility of hydrophilic LMWH and might cause severe conformational changes of angiogenic factors. It was reported that LHT7 formed a rigid helical structure

that facilitated binding to proteins. Therefore, enhanced antiangiogenic effects might be caused by the increased hydrophobicity and reduced flexibility of LMWH [7–9]. In addition, to further improve the oral bioavailability and short half-life of LHT7,

deoxycholic acid, which promotes drug absorption in the intestine, was conjugated and physically complexed with LHT7 to form an oral heparin conjugate (LHTD4). This complex can be effectively and orally administered as an angiogenesis inhibitor [10,11].

Conjugation with hydrophobic groups

Chemical conjugation of drug molecules to polymeric chains has been an effective way to solubilize hydrophobic drugs and prevent drug leakage; however, these approaches tend to be technically challenging and can affect the pharmacological effect of drugs. Physical incorporation of drugs into nanoparticles (NPs) formed by amphiphilic conjugates offers another strategy for drug delivery. Subsequently, hydrophobic drugs are protected inside the micelle via hydrophobic, π-π stacking or other interactions with the core.

The most common LMWH-based NPs are core-shell structural micelles self-assembled by amphiphilic LMWH derivatives (Fig. 2aiii). Generally, amphiphilic LMWH derivatives are prepared by grafting hydrophobic segments, such as all-*trans*-retinoid acid (ATRA) [12], ursolic acid (UA) [13], taurocholate [14], stearyl amine [15], poly($_L$-lactide-*co*-glycolide) [16], deoxycholic acid [17], or cholesterol [18] to LMWH (typical examples are summarized in Table 1). For example, Hou *et al*. developed a novel LMWH-based nanoplatform for the simultaneous delivery of ATRA and paclitaxel (PTX). First, an ATRA prodrug (LHR) was synthesized by directly grafting ATRA to LMWH, then amphiphilic LHR functioning as a polymer carrier further encapsulated the hydrophobic PTX with a high drug loading capacity. The resultant micelle increased the solubility of both chemotherapeutic agents (ATRA and PTX) while being safe for intravenous administration and extending the circulation time of ATRA and PTX. The micelle achieved maximal effects in the inhibition of tumor growth compared with free drug solutions, demonstrating LHR to be a promising multidrug delivery system [19,20]. This example integrates several basic NP construction rules, providing a norm for the simple and effective development of multidrug nanoplatforms.

In addition to nanomicelles, sulfated heparin can also be conjugated with amphiphilic Pluronic copolymers to self-assemble into a nanocomplex. A major advantage of these nanocomplexes is the improved drug encapsulation efficiency and release profile. As a result of the sulfate and carboxylic groups on the heparin chain, positively charged drugs, such as aquated cisplatin [21] and indomethacin [22], can interact with these negatively charged groups. In a study by Tong *et al*., Pluronic (F127) was conjugated with heparin to form nanocomplexes (Hep-F127) at a suitable temperature [21]. Hep-F127 showed a high drug-loading capacity for cisplatin (42.5%, wt/wt), which was significantly higher than a previously reported carboxylated polyamidoamine (PAMAM) dendrimer (25–28%, wt/wt). Moreover, the authors also used Hep-F127 as a carrier for the codelivery of two drugs (e.g., cisplatin and 5-fluorouracil [23]; cisplatin and curcumin [24]) for combination cancer therapy.

Conjugate assembly

LMWH derivatives can also form mixed micelles with other polymers (Fig. 2aiv). To maximize the pharmacological activities of LHT7 and improve its biodistribution profile, Alam *et al*. proposed a novel antiangiogenic therapy that combined strategies of long circulating, passive tumor targeting and antiangiogenesis by developing a new polyelectrolyte complex system comprising LHT7 and protamine. Protamine was able to neutralize the anticoagulation effect of heparin and stabilize the nanocomplex. *In vivo* pharmacokinetic studies showed that the incorporation of protamine significantly increased the bioavailability (2.3 times) of the original preparation and reduced the clearance rate by 3.9 times. Most importantly, the nanocomplex was able to deeply penetrate tumor tissues, indicating that it has good diffusion ability through leaking blood vessels [29]. Furthermore, a cyclic RGDyk moiety with angiogenic endothelial cell and cancer cell dual-targeting ability was chemically conjugated to LHT7 (cRGD-LHT7) to further enhance the antiangiogenetic activity of LHT7 by improving its targeting efficiency. Both *in vitro* and *in vivo* studies revealed the greater antiangiogenetic activity of cRGD-LHT7 compared with original LHT7 [30].

To increase the oral bioavailability of PTX, Pluronic copolymers (F127 or P188) are introduced to form mixed micelles with the above-mentioned LHR conjugation. Compared with LHR, mixed micelles increased the permeability of PTX through the

---

TABLE 1

Examples of LMWH derivatives for cancer therapy

Name	Conjugate	Main benefits	Payload	Main results	Refs
LMWH–ATRA	All-*trans*-retinoid acid	Reduces risk of hemorrhage; increases antiangiogenic effect; synergistic antitumor efficacy with chemo drug	PTX; DOX	Good loading capacities for DOX; effective targeting ability; higher anticancer activity and reduced adverse effects	[19,25]
HDTA	Ursolic acid	Construction of amphipathic skeleton for drug loading	DOX	Excellent oral absorption performance and bioavailability	[26]
LHSA	Stearyl amine	Construction of amphipathic skeleton for drug loading	DOX	High cellular uptake *in vitro*; higher *in vivo* tumor growth inhibition efficacy of DOX-loaded LHSA5 NPs compared with Taxol	[27]
LHT7-ApoPep-1	Taurocholate; apoptosis-homing peptide; ApoPep-1	Increases antiangiogenesis effect	DOX	Increases antiangiogenic and apoptosis-targeting properties; homing to apoptotic cells within tumor	[14]
LHTD4	Taurocholate; deoxycholic acid	Increases binding affinity for VEGF; decreases affinity for antithrombin; blocks multiple proangiogenic factors	–	Retains antiangiogenic activity after oral administration; decreases degree of angiogenesis in lung tissues	[10]
LHsura	Suramin	Increases binding affinity for VEGF; decreases affinity for antithrombin	–	Enhanced antiangiogenic and decreased anticoagulant activity	[28]

rat small intestine. This might be because the Pluronic copolymer inhibited the drug efflux mediated by P-glycoprotein (P-gp), which is widely expressed by intestinal epithelial cells, and cytochrome P450 metabolism (i.e., first-pass effect). Here, because of the low critical micelle concentration (CMC) and retained drug release characteristics, PTX loaded with pluronic/LHR could take full advantage of the P-gp efflux inhibition effect to achieve higher oral bioavailability of PTX (~15–20 times higher) compared with PTX alone [31].

Beyond the classic NP construction strategies, supramolecular assemblies have also enriched the field of LMWH-based NPs. Supramolecular complexes are held together by noncovalent interactions, such as hydrogen bonds, host–guest interactions, electrostatic interactions, and π-π stacking. Multiple noncovalent interactions acting cooperatively with great specificity efficiently bring different pretailored building blocks together [32]. Based on the electrostatic interaction between oppositely charged polymers, polyanion LMWH can spontaneously associate with polycations, such as peptides, chitosan, and polyethyleneimine, to form polyelectrolyte complexes [33]. Likewise, LMWH and its derivatives can cover positively charged liposomes [34], porous nanosilica [35], and dendrimers [36]. LMWH modified with unique elements, such as aromatic substances or cyclodextrin, can form supramolecular nanocomplexes with particular drugs through π-π stacking or host–guest interactions. Thus, pretailored LMWH derivatives might be suitable drug carriers with high drug loading and good stability.

Combination therapy

In addition to cancer-related VTE, it is now widely accepted that LMWH could directly affect the tumor biology by impacting cancer cell adhesion, proliferation, metastasis, and angiogenesis. These broad physiological properties of LMWH provide the basis for its combination with a variety of other antitumor therapies. The most common strategies include the combination of chemotherapeutic drugs to enhance the killing effect on tumors, and the combination of other antitumor angiogenesis drugs to alter the tumor microenvironment through multiple pathways. In addition, LMWH combined with new-emerging treatments, such as nanotheranostics and photodynamic therapy (PDT), have also shown good therapeutic prospects. Although a variety of combination therapies have been explored for antitumor therapy, developing a drug system that is capable of overcoming the complex tumor physiology remains challenging.

Combining LMWH with chemotherapy

Cocktails of therapeutics have received intense research interest to achieve enhanced therapeutic indices *via* combination therapy [37]. In 2014, the LMWH derivative necuparanib (M402) was approved by the US Food and Drug Administration (FDA) in combination with gemcitabine plus albumin-bound PTX for the first-line treatment of metastatic pancreatic cancer. Given that many of the activities of LMWH impact malignant disease, especially its antiangiogenesis and antimetastasis effects, LMWH has potential as a combination therapy to enhance or complement the effects of chemotherapeutics.

The most common strategy used to combine LMWH with chemotherapy is to develop drug delivery systems in which

LMWH derivatives serve as a backbone on which to load the chemotherapeutic drug. Dahmani et al. developed an amphiphilic c(RGDyk)-functional LMWH-gambogic acid (GA) conjugate (cRHG) with specific tumor cells and angiogenic vasculature dual-targeting properties [38]. Unlike most reported angiogenesis and chemotherapy combination therapies, which integrate two different drugs (antiangiogenetic and chemotherapeutic agents) or two different targeting moieties (neovasculature- and tumor cell-targeting ligands) into one system [39,40], this system utilized only one targeting moiety c(RGDyk) with high affinity for $\alpha_v\beta_3$ simultaneously overexpressed in tumor endothelium and tumor cells to realize precise dual-targeting, thereby codelivering GA and LMWH to both tumor cells and the angiogenic vasculature. This multifunctional self-assembled nanosystem inhibited tumor growth either by killing tumor cells after $\alpha_v\beta_3$-mediated internalization or by inhibiting angiogenesis (i.e., VEGF-triggered angiogenesis). In another study, LHT7 was used to coat reduced graphene oxide (rGO) to construct a nano delivery platform. In addition to the increased dispersion stability of rGO in serum, this LHT7-modified nanosheet had strong doxorubicin (DOX) loading capability compared with naked rGO. The resulting LHT-rGO/DOX showed a higher antitumor effect, with a tumor inhibition rate of 92.5%, compared with nondecorated rGO/DOX [41].

In addition to the above-mentioned dual system of LMWH and chemotherapeutic drugs, some researchers have enhanced the antitumor effect through a multicomponent combination therapy system. In a multifunctional polymeric NP developed by Zhang et al., chemotherapy using DOX, ATRA, and LMWH was combined in one platform. LMWH served as a nanocarrier by forming amphiphilic polymers with ATRA while acting as an effective antiangiogenic agent. ATRA induced the differentiation of tumor cells to normal cells, as well as promoting rapid nuclear delivery of DOX because of its nuclear translocation effect, resulting in increased DOX-induced inhibition of DNA biosynthesis. This multifunctional NP with ternary-targeting activities achieved high antitumor efficiency and low toxicity compared with free DOX solutions [25].

Antimetastatic effects of LMWH have been documented *in vivo* and such effects might be related to its interference with cell adhesion molecules [42–44]. Therefore, LMWH and its derivatives could serve as antimetastatic agents to complement chemotherapy to treat metastatic tumors. For example, a LMWH-modified DOX-loaded liposome was developed for the simultaneous delivery of DOX and LMWH for the combined treatment of metastasis. *In vitro* studies showed that LMWH-DOX-Lip inhibited P-selectin-mediated adhesion between B16F10 and activated platelets, thereby successfully inhibiting metastasis; tumor inhibition experiments using a murine lung melanoma model also indicated that tumor growth was suppressed. The authors suggested that LMWH on the surface of liposomes interferes with microemboli formed by tumor cells and platelets in the blood. Subsequently, LMWH-DOX-Lip enters the interior of the tumor tissues, triggering DOX release and inducing tumor cell apoptosis [44,45]. Similarly, comparable with reduction-insensitive liposomes, redox-sensitive LMWH-ss-DOX-Lip can rapidly release DOX and LMWH in the tumor microenvironment, fully exploiting the cytotoxicity of DOX and anti-invasive and antimigration abilities of LMWH. Moreover, studies of heparanase expression further revealed that

LMWH was involved in tumor metastasis attenuation [46]. In another example, a biocompatible copolymer of LMWH and DOX connected by an acid-sensitive hydrazone bond was designed. This NP combining DOX delivery with LMWH antimetastatic capabilities effectively resulted in enhanced antitumor effects and antimetastasis of tumor cells [47].

Combining LMWH with antiangiogenetic therapy

The idea of combining LMWH with other antiangiogenic drugs stems from the complex tumor microenvironment and the process of tumor angiogenesis. It has been well demonstrated that LMWH is an excellent anti-angiogenesis agent because of its competitive inhibition of heparinase and various angiogenic factors. However, there are other enzymes and factors involved in angiogenesis and the effect of LMWH might be counteracted by tumor development. Thus, the combination of LMWH with other antiangiogenic agents with different molecular mechanisms might achieve synergistic effects. The most notable recent success within this line of design could be the combination of LMWH with natural neovascularization inhibitors. Some compounds extracted from natural herbs, such as UA and curcumin, have broad antiangiogenesis activity in different cancers, whereas their poor water solubility and instability limit their pharmacological application.

The chemical grafting of LMWH with UA to prepare NPs was one of the earliest attempts to counteract the drug pharmacokinetic and biodistribution issues while achieving synergistic antiangiogenic effects of the different drugs. In a combination nanosystem named A-LHU (DSPE-PEG-AA-modified LMWH-UA nanodrugs), Li et al. grafted UA with LMWH to develop an amphiphilic conjugate that could self-assemble into nanomicelles. Given that UA exerts its antiangiogenic effects by inhibiting the matrix metalloproteinase (MMP), this alternative mechanism to that of LMWH enables the NP to have multiple targets to inhibit angiogenesis; this expected strong combined antiangiogenic activity was achieved both in vitro and in vivo. Moreover, the nanodrug was further modified with DSPE-PEG-AA for sigma receptor-mediated targeted delivery and the developed A-LHU displayed significant inhibition of tumor growth in melanoma (B16F10 cells)-bearing mice [13,48]. Similarly, Xiao et al. developed LCU (LMWH-curcumin) nanodrugs through chemical coupling of LMWH and curcumin. Not only did the water solubility of curcumin increase from 0.006–0.007 mg/ml to 0.12 mg/ml, enhanced antiangiogenic and antitumor effects were also observed compared to a physical mixture of LMWH and curcumin [49].

Combining LMWH with nanotheranostics

More recently, nanotheranostics, the integration of therapy and imaging in one nanoplatform, has emerged as an efficient way to achieve precise or personalized treatment. Among all nanoplatforms, metallic NPs are ideal candidates owing to their unique features in bioimaging and drug delivery. For example, iron oxide NPs (IONPs) are suitable for MRI and gold (Au)NPs can act as photothermal (PTT) or positron emission tomography/computed tomography (PET/CT) agents [50]. However, these inorganic materials face biocompatibility and stability challenges. Many inorganic NPs are not able to accommodate cargo molecules for nanotheranostics because they are not porous; however, LMWH, as a fraction of endogenous heparin, can endow inorganic particles

with good colloidal stability and bioacceptability, as well as therapeutic functions; thus, using LMWH to modify inorganic NPs could be a promising strategy to construct drug delivery systems. LMWH-coated magnetic NPs could also load protein or chemotherapeutics drug through electrostatic, hydrophobic, or other attractions [51,52].

Groult et al. developed IONPs coated with different heparins with distinct anticoagulant:antiheparanase activity ratios for specific bioactivity and bioimaging applications. Thus, NPs are promising for thromboembolism clinical MRI diagnosis and antitumor therapeutic strategies [53]. LMWH-deoxycholic acid amphipathic polymers, adsorbed in the small intestine specifically through bile acid transporters in the ileum, were used to load near-infrared quantum dots (QD) for biomedical imaging. The resulting stable NPs were successfully absorbed through oral administration, thus enabling real-time observation of the pharmacokinetics of the NPs [17,54].

There are other nanotheranostic platforms in addition to inorganic platforms. Redox-responsive therapy diagnostic NPs are nano drug delivery systems using LMWH as the backbone. Here, chloroe-6 (Ce-6) and α-tocopherol succinate (TOS) are conjugated to LMWH via a redox-sensitive linker. Following self-assembling and PTX encapsulation, the formed NPs can realize near-infrared (NIR) imaging-guided combined photodynamic/tumor chemotherapy. In addition, these NPs maintained the antimetastatic activity of LMWH. This design successfully incorporated chemotherapy, PDT, as well as the antimetastatic potential of LMWH, demonstrating the enormous potential of intelligent NIR imaging-guided tumor combination therapy [55].

Other approaches

Following LHT7 administration, hypoxia-mediated cyclo-oxygenase 2 (COX-2) overexpression and macrophage recruitment were observed in tumor tissues; thus, it was reasonable to combine LHT7 with a COX-2 inhibitor (celecoxib), which can inhibit the inflammatory reactions induced by hypoxia and alter vascular stabilization. Both COX-2 overexpression and macrophage recruitment were well controlled by this combined therapy. In addition, both in vitro and in vivo tumor vessel formation and structure were further enhanced, demonstrating that the therapeutic effect of LHT7 was strengthened by COX-2 inhibition [56].

Given that angiogenesis and apoptosis are often accompanied by tumorigenesis, it should be reasonable to develop a cocktail therapy that simultaneously blocks these processes. For example, one study introduced an apoptosis-homing peptide (ApoPep-1) to conjugate with the LHT7 (LHT7-ApoPep-1), in which antiangiogenic and apoptosis-targeting properties were combined for cancer therapy; LHT7-ApoPep-1 successfully delayed MDA-MB-231 tumor growth compared with the monotherapy (LHT7) [14].

Recently, heparin-based self-assembly NPs were used for PDT, a clinically available therapeutic option whereby cancer cells are destroyed by a combination of light and photosensitizers (PSs). In such cases, heparin usually functions as backbone either by forming heparin–PS conjugates or by physical incorporation of PS with heparin amphiphilic polymers. Antiangiogenic treatment was found to potentiate PDT responsiveness by attenuating the angiogenic actions of VEGF, which is increased in PDT-treated tumors [57]. In addition, almost all PDT effects are oxygen dependent,

such that photosensitization is typically restricted in anoxic tumor regions. PDT was also found to cause microvascular collapse, further leading to tumor hypoxia and anoxia. The consumption of oxygen after PDT also leads to tumor deterioration [58]. Therefore, as well as serving as a backbone, LMWH and its derivatives might take advantage of both antiangiogenesis and vessel normalization, resulting in

remission in tumor hypoxia, synergistic efficiencies, and improved prognosis.

Concluding remarks

Although mechanisms underlying the anticancer effects of LMWH have been discovered over the past 20 years at an increasing rate,
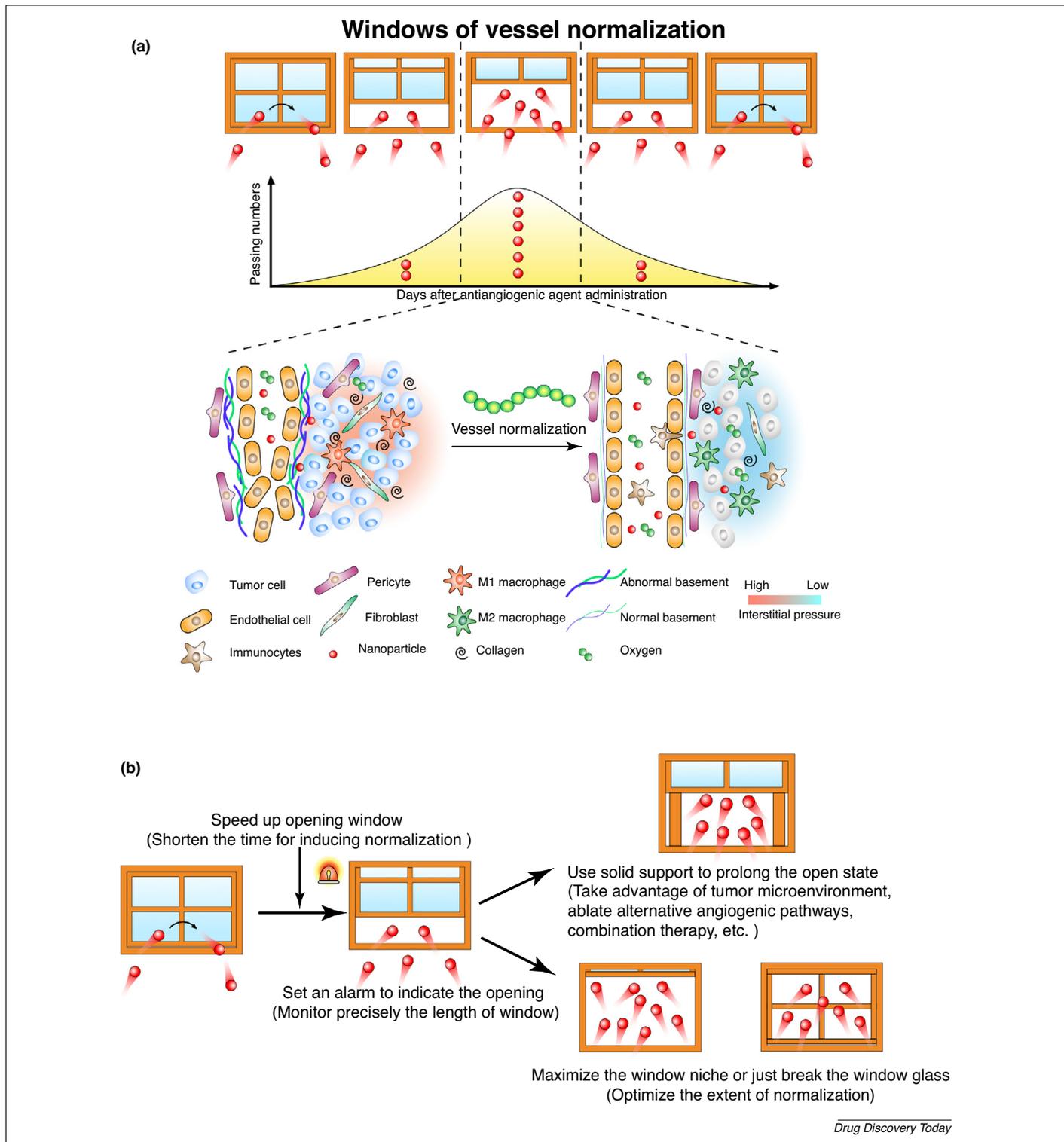
FIGURE 3

Vessel normalization as a potential approach. **(a)** Improved nanodrug targeting and retention within normalization window; **(b)** strategies to optimize normalization-based therapies.

our understanding of the effect of LMWH on tumor biology is incomplete. In particular, the proposal and development of the vascular normalization theory greatly impacted the existing pharmacological mechanisms of antiangiogenic drugs, such as LMWH. This theory holds that, in contrast to destroying tumor vessels, antiangiogenic agents restore tumor vessels and normalize the abnormal structure and function of tumor vessels, thus making them more efficient for oxygen and drug delivery (Fig. 3a). Inspired by this theory, research has focused on the antiangiogenic mechanisms of heparin and LMWH. Studies have shown that heparin molecules are able to dissolve microthrombi comprising fibrin and other plasma proteins in the tumor microenvironment, thereby reducing the interstitial pressure, which could also benefit the deeper penetration of drugs into the tumor [59,60]. Preclinical studies showed that LMWH, as a potent VEGF inhibitor, could induce vessel normalization transiently during antiangiogenic therapy [61,62]. Furthermore, recently published research utilized this characteristic of LMWH to construct a nanodrug with potent vascular normalization-promoting ability, achieving encouraging results in the modulation of the tumor microenvironment and drug delivery with tumor tissues [63].

Collectively, researchers could take advantage of the vessel normalization potential of LMWH as an antitumor strategy as well as a combination therapy. Studies have shown that vascular normalization can promote the infiltration of immune cells into tumor tissues, enhancing the sensitivity of the tumor cells to immunotherapy [64,65]. These findings provide a theoretical basis for future anticancer combinatorial therapies. As an inducer of tumor vessel normalization, LMWH might also achieve synergistic effects in combination with other immunotherapies.

However, all these approaches present both opportunities and challenges. A major challenge for this new combination therapy is the optimization of dose and schedule to create a sustained and controllable window of vessel normalization allowing chemotherapeutics maximal access to tumor cells (Fig. 3b). Thus, further research into the combination therapy of LMWH and derivatives with chemotherapy is required to realize the full potential of this therapeutic strategy.

References

1 Mousa, S.A. and Petersen, L.J. (2009) Anti-cancer properties of low-molecular–weight heparin: preclinical evidence. *Thromb. Haemost.* 102, 258

2 McCrae, K.R. *et al.* (2001) Platelets: an update on diagnosis and management of thrombocytopenic disorders. *Hematology* 282–305

3 Park, J. *et al.* (2015) Chemical conjugate of low molecular weight heparin and suramin fragment inhibits tumor growth possibly by blocking VEGF165. *Mol. Pharm.* 12, 3935–3942

4 Chung, S.W. *et al.* (2012) Potentiation of anti-angiogenic activity of heparin by blocking the ATIII-interacting pentasaccharide unit and increasing net anionic charge. *Biomaterials* 33, 9070–9079

5 Muñoz, E.M. and Linhardt, R.J. (2004) Heparin-binding domains in vascular biology. *Arterioscler. Thromb. Vasc. Biol.* 24, 1549

6 Park, K. *et al.* (2006) Heparin-deoxycholic acid chemical conjugate as an anticancer drug carrier and its antitumor activity. *J. Control. Release* 114, 300–306

7 Jin, W.P. *et al.* (2010) High antiangiogenic and low anticoagulant efficacy of orally active low molecular weight heparin derivatives. *J. Control. Release* 148, 317–326

8 Lee, E. *et al.* (2009) Polyproline-type helical-structured low-molecular weight heparin (LMWH)–taurocholate conjugate as a new angiogenesis inhibitor. *Int J. Cancer* 124, 2755–2765

9 Park, K. *et al.* (2007) Antiangiogenic effect of bile acid acylated heparin derivative. *Pharm. Res.* 24, 176–185

10 Kim, J.Y. *et al.* (2015) Antiangiogenic and anticancer effect of an orally active low molecular weight heparin conjugates and its application to lung cancer chemoprevention. *J. Control. Release* 199, 122–131

11 Alam, F. *et al.* (2014) Oral delivery of a potent anti-angiogenic heparin conjugate by chemical conjugation and physical complexation using deoxycholic acid. *Biomaterials* 35, 6543–6552

12 Yoo, S.H. Preparation of aqueous clear solution dosage forms with bile acids. WO2001056547B1.

13 Li, Y. *et al.* (2016) Sigma receptor-mediated targeted delivery of anti-angiogenic multifunctional nanodrugs for combination tumor therapy. *J. Control. Release* 228, 107

14 Bae, S.M. *et al.* (2013) An apoptosis-homing peptide-conjugated low molecular weight heparin-taurocholate conjugate with antitumor properties. *Biomaterials* 34, 2077–2086

15 Kim, D.H. *et al.* (2014) Preparation and characterization of self-assembled nanoparticles based on low-molecular-weight heparin and stearylamine conjugates for controlled delivery of docetaxel. *Int. J. Nanomed.* 9, 5711

16 Jeon, O. *et al.* (2006) Long-term and zero-order release of basic fibroblast growth factor from heparin-conjugated poly(L-lactide-co-glycolide) nanospheres and fibrin gel. *Biomaterials* 27, 1598–1607

17 Khatun, Z. *et al.* (2012) Oral delivery of near-infrared quantum dot loaded micelles for noninvasive biomedical imaging. *ACS Appl. Mater. Interface* 4, 3880

18 Sun, H. *et al.* (2018) Development of low molecular weight heparin based nanoparticles for metastatic breast cancer therapy. *Int. J. Biol. Macromol.* 112, 343–355

19 Hou, L. *et al.* (2011) Low molecular weight heparin-all-trans-retinoid acid conjugate as a drug carrier for combination cancer chemotherapy of paclitaxel and all-trans-retinoid acid. *Carbohydr. Polym.* 86, 1157–1166

20 Lin, H. *et al.* (2012) Pharmacokinetics of a paclitaxel-loaded low molecular weight heparin-all-trans-retinoid acid conjugate ternary nanoparticulate drug delivery system. *Biomaterials* 33, 5431–5440

21 Tong, N.-A.N. *et al.* (2016) Aquated cisplatin and heparin-pluronic nanocomplexes exhibiting sustainable release of active platinum compound and NCI-H460 lung cancer cell antiproliferation. *J. Biomater Sci. Polym. Ed.* 27, 709–720

22 Choi, J.H. *et al.* (2011) Self-assembled nanogel of pluronic-conjugated heparin as a versatile drug nanocarrier. *Macromol. Res.* 19, 180–188

23 Tong, N.-A.N. *et al.* (2018) Thermosensitive heparin-Pluronic® copolymer as effective dual anticancer drugs delivery system for combination cancer therapy. *Int. J. Nanotechnol.* 15, 174–187

24 Nguyen, N. *et al.* (2018) Synergic activity against MCF-7 breast cancer cell growth of nanocurcumin-encapsulated and cisplatin-complexed nanogels. *Molecules* 23, 3347

25 Zhang, T. *et al.* (2015) Combination chemotherapy of doxorubicin, all-trans retinoic acid and low molecular weight heparin based on self-assembled multi–functional polymeric nanoparticles. *Nanotechnology* 26, 145101

26 Khatun, Z. *et al.* (2013) Oral delivery of taurocholic acid linked heparin-docetaxel conjugates for cancer therapy. *J. Control. Release* 170, 74–82

27 Kim, D.-H. *et al.* (2014) Preparation and characterization of self-assembled nanoparticles based on low-molecular-weight heparin and stearylamine conjugates for controlled delivery of docetaxel. *Int. J. Nanomed.* 9, 5711

28 Jooho, P. *et al.* (2015) Chemical conjugate of low molecular weight heparin and suramin fragment inhibits tumor growth possibly by blocking VEGF165. *Mol. Pharm.* 12, 3935–3942

29 Farzana, A. *et al.* (2015) Functionalized heparin-protamine based self-assembled nanocomplex for efficient anti-angiogenic therapy. *J. Control. Release* 197, 180–189

30 Adulnirath, A. *et al.* (2012) Cyclic RGDyk-conjugated LMWH-taurocholate derivative as a targeting angiogenesis inhibitor. *J. Control. Release* 164, 8–16

31 Dahmani, F.Z. *et al.* (2012) Enhanced oral bioavailability of paclitaxel in pluronic/LHR mixed polymeric micelles: preparation, *in vitro* and *in vivo* evaluation. *Eur. J. Pharm. Sci.* 47, 179

32 Liu, K. *et al.* (2013) 25th Anniversary Article: reversible and adaptive functional supramolecular materials: 'noncovalent interaction' matters. *Adv. Mater.* 25, 5530–5548

33 Park, J. *et al.* (2017) Self-assembled nanocomplex of PEGylated protamine and heparin–suramin conjugate for accumulation at the tumor site. *Int. J. Pharm.* 535, 38

34 Li, H. *et al.* (2017) Effects of surface charge of low molecular weight heparin modified cationic liposomes on drug efficacy and toxicity. *Drug Dev. Commun.* 43, 1163–1172

35 Thi, T.T.N. *et al.* (2017) Hierarchical self-assembly of heparin-PEG end-capped porous silica as a redox sensitive nanocarrier for doxorubicin delivery. *Mater. Sci. Eng. C* 70, 947–954

36 Thanh, V.M. *et al.* (2018) Low systemic toxicity nanocarriers fabricated from heparin-mPEG and PAMAM dendrimers for controlled drug release. *Mater. Sci. Eng. C* 82, 291–298

37 Liang, K. *et al.* (2014) Peptide-tunable drug cytotoxicity via one-step assembled polymer nanoparticles. *Adv. Mater.* 26, 2398–2402

38 Dahmani, F.Z. *et al.* (2016) Multifunctional polymeric nanosystems for dual-targeted combinatorial chemo/angiogenesis therapy of tumors. *Adv. Healthc. Mater.* 5, 1447

39 Huang, S. *et al.* (2013) Tumor-targeting and microenvironment-responsive smart nanoparticles for combination therapy of antiangiogenesis and apoptosis. *ACS Nano* 7, 2860–2871

40 Gao, H. *et al.* (2014) Tumor cells and neovasculature dual targeting delivery for glioblastoma treatment. *Biomaterials* 35, 2374–2382

41 Shim, G. *et al.* (2014) Reduced graphene oxide nanosheets coated with an anti-angiogenic anticancer low-molecular-weight heparin derivative for delivery of anticancer drugs. *J. Control. Release* 189, 80–89

42 Mousa, S.A. *et al.* (2006) Anti-metastatic effect of a non-anticoagulant low-molecular-weight heparin versus the standard low-molecular-weight heparin, enoxaparin. *Thromb. Haemost.* 96, 816

43 Kragh, M. and Loechel, F. (2005) Non-anti-coagulant heparins: a promising approach for prevention of tumor metastasis (review). *Int. J. Oncol.* 27, 1159

44 Casu, B. *et al.* (2008) Non-anticoagulant heparins and inhibition of cancer. *Pathophysiol. Thromb. Haemost.* 36, 195–203

45 Chen, Y. *et al.* (2015) A low-molecular-weight heparin-coated doxorubicin-liposome for the prevention of melanoma metastasis. *J. Drug Target.* 23, 335

46 Tian, J. *et al.* (2016) Reduction responsive modification induced higher efficiency for attenuation of tumor metastasis of low molecular weight heparin functionalized liposomes. *RSC Adv.* 6, 49250–49262

47 Mei, L. *et al.* (2017) Polymer-drug nanoparticles combine doxorubicin carrier and heparin bioactivity functionalities for primary and metastatic cancer treatment. *Mol. Pharm.* 14, 513–522

48 Cheng, W. *et al.* (2017) Anti-angiogenic activity and antitumor efficacy of amphiphilic twin drug from ursolic acid and low molecular weight heparin. *Nanotechnology* 28 075102

49 Xiao, Y. *et al.* (2018) Dose-reduction antiangiogenic curcumin-low molecular weight heparin nanodrugs for enhanced combinational antitumor therapy. *Eur. J. Pharm. Sci.* 119, 121–134

50 Ma, Y. *et al.* (2016) Cancer-targeted nanotheranostics: recent advances and perspectives. *Small* 12, 4936–4954

51 Zhang, J. *et al.* (2013) Long-circulating heparin-functionalized magnetic nanoparticles for potential application as a protein drug delivery platform. *Mol. Pharm.* 10, 3892–3902

52 Chertok, B. *et al.* (2011) Magnetically-enabled and MR-monitored selective brain tumor protein delivery in rats via magnetic nanocarriers. *Biomaterials* 32, 6245–6253

53 Groult, H. *et al.* (2017) Family of bioactive heparin-coated iron oxide nanoparticles with positive contrast in magnetic resonance imaging for specific biomedical applications. *Biomacromolecules* 18, 3156–3167

54 Khatun, Z. *et al.* (2012) Imaging of the GI tract by QDs loaded heparin-deoxycholic acid (DOCA) nanoparticles. *Carbohydr. Polym.* 90, 1461–1468

55 Yang, X. *et al.* (2018) Development of redox-responsive theranostic nanoparticles for near-infrared fluorescence imaging-guided photodynamic/chemotherapy of tumor. *Drug Deliv.* 25, 780

56 Kim, J.Y. *et al.* (2015) Enhanced anti-angiogenic effect of low molecular weight heparin-bile acid conjugates by co-administration of a selective COX-2 inhibitor. *Pharm. Res.* 32, 1–10

57 Ferrario, A. *et al.* (2000) Antiangiogenic treatment enhances photodynamic therapy responsiveness in a mouse mammary carcinoma. *Cancer Res.* 60, 4066–4069

58 Dolmans, D.E. *et al.* (2003) Photodynamic therapy for cancer. *Nat. Rev. Cancer* 3, 380–387

59 Lebeau, B. *et al.* (2015) Subcutaneous heparin treatment increases survival in small cell lung cancer. 'Petites Cellules' Group. *Cancer* 74, 38–45

60 Pfankuchen, D.B. *et al.* (2015) Low molecular weight heparin tinzaparin antagonizes cisplatin resistance of ovarian cancer cells. *Biochem. Pharmacol.* 97, 147

61 Debergh, I. *et al.* (2015) Microvascular effects of the low molecular weight heparins in a colorectal xenograft model: an intravital microscopy study. *J. Surg. Res.* 194, 488–495

62 Debergh, I. *et al.* (2010) The low-molecular-weight heparin, nadroparin, inhibits tumour angiogenesis in a rodent dorsal skinfold chamber model. *Br. J. Cancer* 102, 837

63 Du, S. *et al.* (2019) Attempts to strengthen and simplify the tumor vascular normalization strategy using tumor vessel normalization promoting nanomedicines, Bio. *Mater. Sci.* 7, 1147–1160

64 Noman, M.Z. *et al.* (2015) Hypoxia: a key player in antitumor immune response. A review in the theme: cellular responses to hypoxia. *Am. J. Physiol. Cell Physiol.* 309, 569–579

65 Tian, L. *et al.* (2017) Mutual regulation of tumour vessel normalization and immunostimulatory reprogramming. *Nature* 544, 250–254