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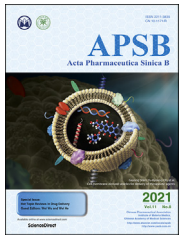

REVIEW

Emerging nanomedicine-based therapeutics for hematogenous metastatic cascade inhibition: Interfering with the crosstalk between “seed and soil”

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Premetastatic niche

Abstract Despite considerable progresses in cancer treatment, tumor metastasis is still a thorny issue, which leads to majority of cancer-related deaths. In hematogenous metastasis, the concept of “seed and soil” suggests that the crosstalk between cancer cells (seeds) and premetastatic niche (soil) facilitates tumor metastasis. Considerable efforts have been dedicated to inhibit the tumor metastatic cascade, which is a highly complicated process involving various pathways and biological events. Nonetheless, satisfactory therapeutic outcomes are rarely observed, since it is a great challenge to thwart this multi-phase process. Recent advances in nanotechnology-based drug delivery systems have shown great potential in the field of anti-metastasis, especially compared with conventional treatment methods, which are limited by serious side effects and poor efficacy. In this review, we summarized various factors involved in each phase of the metastatic cascade ranging from the metastasis initiation to colonization. Then we reviewed current approaches of targeting these factors to stifle the metastatic cascade, including modulating primary tumor microenvironment, targeting circulating tumor cells, regulating premetastatic niche and eliminating established metastasis. Additionally, we highlighted the multi-phase targeted drug delivery systems, which hold a better chance to inhibit metastasis. Besides, we demonstrated the limitation and future perspectives of nanomedicine-based anti-metastasis strategies.

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1. Introduction

Today, tumor metastasis, the spread of tumor cells from primary tumor to distant organs, has become the major cause for approximately 90% of cancer-related deaths, with poor therapeutic efficacy and prognosis. Worse still, over half of the patients have already presented metastatic diseases by the time the cancer is diagnosed[1]. Major clinical treatments like surgery, chemotherapy and radiotherapy not only cannot eradicate metastasis, but may increase the risks of recurrence, leading to exacerbated micrometastasis and formulation of new metastatic foci[2,3]. Thus, finding effective therapeutic strategies to prevent and inhibit cancer metastasis is vitally imperative. Unfortunately, the highly sophisticated metastatic cascade, which involves various complicated pathways, put forward higher bars for the anti-metastasis drug development. Current anti-metastasis strategies are mainly divided into two parts, preventing tumor cell dissemination and inhibiting existing metastasis, both of which are far from optimal due to the non-specific toxicities and limited efficacy[4].

Despite enormous challenges, considerable efforts have been dedicated to unveil the underlying molecular and cellular mechanisms of cancer metastasis progression in the past decades. More and more signal pathways, metastasis-associated factors and new concepts have been introduced to expand our understanding of tumor metastasis[5,6]. With fresh insight into metastasis, encouraging progress has been made to thwart it *via* nanotechnology-based drug delivery systems (nano-DDSs), which have attracted considerable attention in the past two decades. Various nanosystems such as organic nanoparticles, inorganic nanoparticles, micelles, liposomes and biomimetic nanoparticles have shown significant efficacy in a wide range of diseases[7]. By using nanoparticles (NPs) as vehicles, anticancer agents such as chemotherapeutics, photodynamic agents, small molecule inhibitors, protein, peptides as well as nucleic acid can attain enhanced therapeutic potential and minimized influence to normal tissue[8]. With preferential size and prolonged circulation, NPs are capable of penetrating leaky vasculature and impaired lymphatics into tumor site to fulfill enhanced permeability and retention (EPR)[9]. Of note, the EPR effect is corroborated to be inter- and intra-individually heterogeneous, accounting for the unsatisfactory outcomes in clinical trials. A better understanding of the pathophysiological characteristics of the lesion, more rational design of nano-DDS and optimization of clinical trials design are urgently needed to address the problem[10]. In addition, by virtue of advanced nanotechnology, NPs are able to achieve active targeting, which facilitates tumor-selective accumulation of payloads, either by their stimuli-responsive nano-structures[11], or by the surface modified ligands that specifically recognize the biomarkers on tumor cells[12]. Due to the unique chemical and physical properties, a single nanoparticle may simultaneously perform both diagnostic and therapeutic functions[13].

For a long time, the development of anti-metastasis drugs has been an unpopular area, since validated therapeutic outcomes are seldom observed in clinical trials. One possible reason is that monotherapy is not enough to terminate the metastatic cascade and even combination therapy cannot guarantee the effective concentration of several drugs at the lesion[14]. Besides, some drugs that exhibited significant activity in preclinical models were restricted by their poor pharmacokinetic properties, resulting in failure in clinical trials[15,16]. Even some approved drugs have been identified to induce metastasis in preclinical models, possibly as a result of systemic adverse reactions, such as the wound healing-type recovery and concomitant pro-metastasis factors[17,18]. The emergence of nano-DDS sheds new light to solve the above thorny problems. For instance, encapsulating drugs into nanocarriers can minimize drug exposure to non-target tissue, while enhancing enrichment in target sites and promoting long circulation. Moreover, some co-delivery systems are able to realize precise and controlled release of various therapeutic agents to thwart multiple metastasis-associated pathways simultaneously. However, few review articles focus on the use of nanomedicine to fight against the notorious tumor metastatic cascade.

2. The metastatic cascade: implication for targeting

In 1889, Steven Paget proposed the "seed and soil" theory, suggesting that while cancer cells spread to the whole body, they prefer to form metastasis in specific distant organs, which provide favorable environments for the survival of tumor cells[19]. According to Paget's theory, the seeds (tumor cells with metastatic potential) breed in the primary soil (tumor microenvironment) and disseminate to circulation. Finally, some of them colonize the secondary soil (a permissive environment that supports the formation of metastatic foci) and form metastasis[20,21]. Overall, tumor metastasis can be concluded into complex communication among these three individual factors (Fig. 1). Therapeutic approaches that interrupt the interplay between the three factors stand a good chance to stifle the metastatic cascade.

The multistage metastatic cascade can be summarized into several steps: 1) primary tumor cells get released from adhesive attachments and invade through basement membrane; 2) undergo epithelial mesenchymal transition (EMT) and cross extracellular matrix (ECM) as well as adjacent stroma; 3) invasive carcinoma cells penetrate blood vessels and enter circulation (intravasation); 4) circulating tumor cells (CTCs) manage to survive and disseminate; 5) survived tumor cells get arrested at distant organs and escape from vasculature (extravasation); 6) adapt the microenvironment of foreign tissue; 7) restore the ability of malignant proliferation and form metastasis (metastatic colonization).

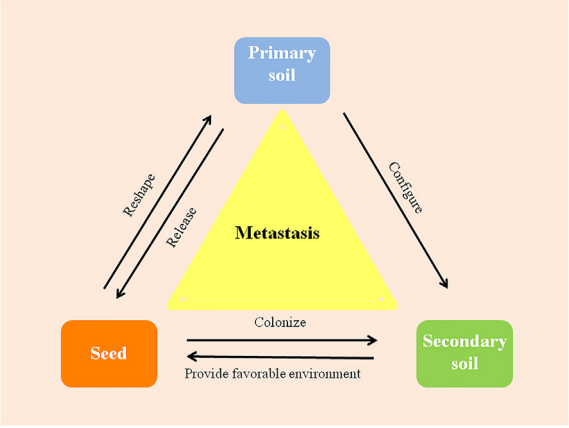

Figure 1 A schematic diagram of the triangular relationship among primary soil, seed, and secondary soil. The primary soil gives birth to the seeds, which in turn reshape the primary soil to facilitate metastatic initiation. Besides, the secondary soil is pre-configured by primary soil into favorable environment for the colonization of disseminated seeds.

Obviously, tumor metastasis is a sophisticated chain reaction composed of series of biological events, wherein seeds detach from primary soil, followed by transporting through circulation and colonizing the secondary soil[22] (Fig. 2).

3. Targeting the primary soil by nano-DDS: Preventing the metastatic dissemination

The primary soil, which refers to the primary tumor microenvironment (PTME), plays an essential role in the metastatic cascade. The PTME is composed by a complicated network of immune cells, vasculature, stromal cells, extracellular matrix (ECM), as well as hypoxic and acidic physical environment. These cell and non-cell components directly or indirectly promote tumor invasion and migration towards vessels *via* various pathways mediated by chemokines, matrix metalloproteinase (MMP) and growth factors[23−25]. Compared with tumor cells, the PTME is genetically more stable and more accessible, containing many attractive targets. Therefore, reshaping the PTME to restore the homeostatic microenvironment is a promising approach to stifle the earliest steps of metastasis initiation. By encapsulating various therapeutic agents and their intrinsic properties, NPs may serve a purpose to restrain the invasion and migration of tumor cells, thus preventing the seeds detaching from normalized primary soil (Table 1[26-59]).

3.1. *Modulating metabolic microenvironment*

Among various physiological features involved in PTME, hypoxia and acidosis are two interrelated key factors that provide beneficial niches for tumor progression and metastasis. As tumor grows to a specific size, the oxygen purveyed by surrounding vessels is insufficient and gradually depleted, thus causing a hypoxic microenvironment. To maintain oxygen homeostasis, HIF signaling, which is mediated by the hypoxia-inducible transcription factors HIF-1 and HIF-2, is activated to promote oxygen delivery and coordinate cellular adaptation to oxygen tensions. The HIF signaling participates in almost every step of metastatic cascade, including invasion, EMT, angiogenesis and establishment of premetastatic niche[60]. Indeed, it has been demonstrated in experimental models that up-regulated HIF would increase the metastatic potential of tumor cells[61,62]. In addition, hypoxia is also closely related to the resistance of chemotherapy, radiation therapy and photodynamic therapy (PDT), resulting in compromised efficacy and poor prognosis[63,64].

Basically, the state-of-art strategies that regulate hypoxia and mitigate downstream negative effects mainly focus on increasing *in situ* generation of oxygen, enhancing tumor-targeted oxygen delivery, decreasing oxygen consumption and silencing the HIF pathway. Yang et al.[26] developed an intelligent biodegradable hollow manganese dioxide (H−MnO_2) nano-platform loaded with chlorine e6, a photodynamic agent, and doxorubicin. The MnO_2 nanostructure could not only achieve controlled release of drug in response to H^+ and glutathione (GSH) within the tumor microenvironment (TME), but also decompose tumor endogenous H_2O_2 to relieve hypoxia microenvironment. The Mn^{2+} ions generated by reaction of MnO_2 with H^+ or GSH could enhance tumor-specific imaging and detection. In addition, this multifunctional nano-system was capable of reversing the immunosuppressive microenvironment and trigger anti-tumor immune responses, especially when combined with immune checkpoint blockage. *In vivo* experiments, 4T1 cells were subcutaneously inoculated into the left and right flanks of mice to simulate primary and metastatic distant tumors. The primary tumors were treated with light irradiation while the distant tumors were deprived of light-induced PDT. Impressively, after combining chemo-photodynamic therapy with PD-L1 blockade therapy on primary tumors, the growth of distant tumors were also effectively suppressed whereas the other groups were not significantly affected,

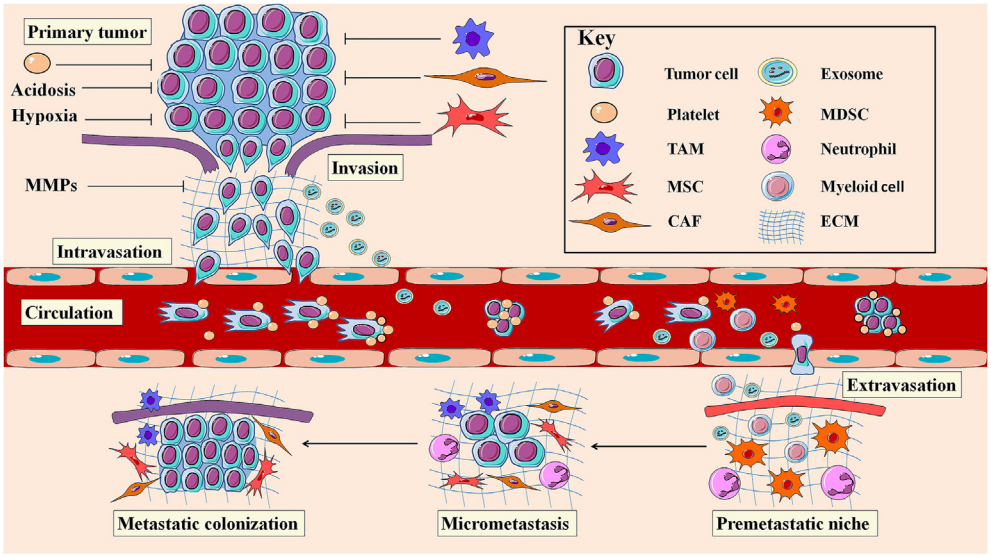

Figure 2 The process of metastasis starts with local invasion [assisted by tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC), platelets, cancer associated fibroblasts (CAFs), ECM (loosened by matrix metalloproteinases (MMPs), etc.], followed by intravasation, circulation (shield by platelets), extravasation and colonization of distant organs. Notably, prior to the metastatic dissemination, the susceptible distant organs have already been reshaped by myeloid-derived suppressor cells (MDSC), myeloid cells, tumor-derived exosomes, neutrophils, mesenchymal stem cell (MSC), etc.

Table 1 Current nanomedicine-based anti-metastasis strategies of targeting primary tumor microenvironment.

Target	Role in the metastatic cascade	Strategy	Advantage	Ref.
Hypoxia	Promote invasion, EMT, angiogenesis and formation of PMN	Increase *in situ* generation of oxygen	Relieve oxygen tensions	26
		Enhance tumor-targeting oxygen delivery	Inhibit HIF signal pathways	27
		Decrease oxygen consumption	Reduce the metastatic potential of tumor cells	28
		Silence the HIF pathway	Reverse the immunosuppressive microenvironment	29,30
Acidosis	Enhance local invasion, migration and angiogenesis	Use basic materials or drugs to neutralize acidity	Restrain the invasion and migration of tumor cells	31,32
		Restrain the cellular efflux of lactate		33
		Relieve hypoxia		34
Vasculature	Promote intravasation	Anti-angiogenesis	Suppress intravasation	35,36
		Vascular normalization	Reduce vascular permeability	37
		Combine anti-angiogenesis with cytotoxic therapeutic approaches		38,39
Platelets	Enhance angiogenesis and vascular permeability	Targeted depletion of intratumoral platelets	Enhance the accumulation of therapeutic agents	40
	Protect CTCs	Cut off the interactions between tumor cells and platelets	Suppress tumor invasion	41
			Reduce the survival rate of CTCs	41
Extracellular matrix	Promote invasion and migration	Enhance cell−cell and cell-EMC adhesion	Inhibit metastatic initiation	42
		Inhibit MMPs		43
		Reinforce the structure of ECM		44
Cancer associated fibroblasts	Secrete metastasis associated molecules	Deplete CAFs locally	Inhibit tumor cell invasion and migration	45
	Promote tumor cells migration	Transform CAFs into inactivated phenotype		46−49
	Reshape ECM			46−49
Tumor associated macrophages	Enhance the motility of tumor cells	Selectively kill M2-like macrophages	Reverse the immune suppressive microenvironment	50
	Immunosuppressive	Repolarize M2-like macrophages into M1 phenotype	Inhibit metastatic initiation	51,52
	Loosen the structure of ECM			51,52
	Trigger EMT			51,52
EMT	Enhance motility and contractility of tumor cells	Regulate EMT associated pathways	Inhibit metastatic initiation	53−55
	Promote the expression of VEGFs and MMPs	Eliminate EMT-type cells	Prevent metastatic dissemination	56
Cancer stem cells	The most tenacious seeds within PTME	Develop specific and effective targeting tools	Eradicate primary tumor	57
		Target stem-like tumor cells	Eliminate the underlying hazard of metastasis	53,57,58
		Synergetic combination therapy	Prevent tumor metastasis	59

suggesting that H−MnO_2 nano-platform was a promising tool to prohibit metastasis (Fig. 3). To achieve efficient tumor-targeted oxygen delivery, Zhou et al.[27] constructed a two-stage oxygen delivery system based on perfluorotributylamine, one member of perfluorocarbon compounds with strongest platelets inhibition ability. The first stage of oxygen supply came from the physically adsorbed oxygen of perfluorotributylamine and the second stage owed to the increased red blood cell infiltration into tumors by virtue of platelet inhibition. In another case, an HIF-1α-knock-down strategy was proposed by Li et al.,[29] who targeted CRISPR/Cas9 system and PTX into tumor spheroids *via* R8-dGR peptide modified cationic liposomes. The downstream metastasis-related molecules such as VEGF and MMP-9 were also down-regulated and the metastasis of pancreatic cancer was successfully suppressed.

In response to hypoxia, cancer cells will undergo a metabolic switch to intensify anerobic glycolysis, which increases the production of lactic acid, leading to accumulation of H^+ in the extracellular space and significant acidification of the tissue. It was identified that local tumor pH distribution was heterogeneous and the regions of lowest pH displayed highest invasiveness[65]. This phenomenon can be explained by the enhanced proteolytic activity of matrix metalloproteinases (MMPs) and up-regulated expression of cathepsin B, both of which function to degrade and loosen the structure of extracellular matrix.

The most direct way to neutralize the acidic environment is to utilize alkaline substances[31]. Indeed, it has been also reported that tumor-targeted delivery of sodium bicarbonate could elevate the intratumoral pH and promote cellular uptake of

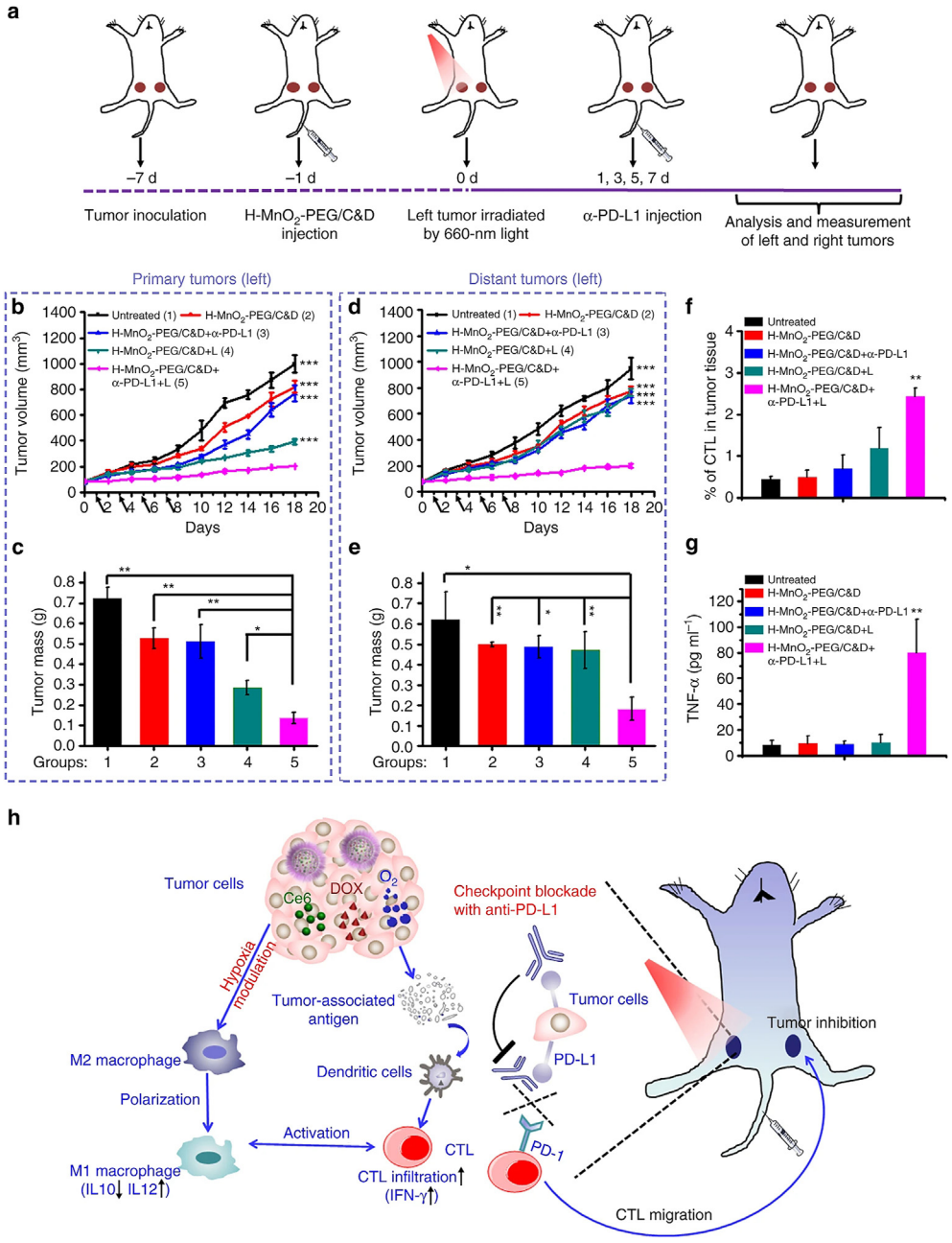

Figure 3 The abscopal effect of H−MnO2-PEG/C&D in combination with anti-PD-L1 (α-PD-L1) checkpoint blockade. Schematic illustration of H−MnO2-PEG/C&D and anti-PD-L1 combination therapy. Primary (b) and distant (d) tumors growth curves of different groups of mice after various treatments indicated. Error bars are based on SEM (six mice per group). The arrows represent the time points of anti-PD-L1 administration. Average weights of primary (c) and distant (e) tumors collected from mice 18 days after initiation of various treatments. (f) CTL infiltration in distant tumors. $CD3^+CD8^+$ cells were defined as CTLs. (g) The production of TNF-α in sera of mice determined on the 9th day post various treatments. (h) The proposed mechanism of anti-tumor immune responses induced by H−MnO2-PEG/C&D in combination with anti-PD-L1 therapy. *P* values were calculated by Tukey's post-test (***$P < 0.001$, **$P < 0.01$, or *$P < 0.05$). Reprinted with permission from Ref. 26.

weakly basic doxorubicin[32]. Recently, Chen et al.[33] managed to interfere with lactate metabolism of tumor cells *via* MnO_2-coated mesoporous silicon nanoparticles (MSNs), wherein the co-encapsulated metformin (Me) and fluvastatin sodium (Flu) synergistically promoted the production of lactate and concurrently restrained their efflux, resulting in cancer cell death of acidosis. Interestingly, it was observed that the migration ability of tumor cells was restricted in the wound-healing test and transwell invasion assay, suggesting that modulating the acidic microenvironment could reduce the metastatic potential of tumor cells. Additionally, as we discussed above, hypoxia and acidosis are interrelated factors, so relieving hypoxia may also contribute to alleviate the acidic microenvironment in principle[34].

3.2. *Targeting tumor vasculature*

During the rapid progress of tumor growth, a vascular network that purveys nutrients for tumor cells is essential. To meet their increasing needs for proliferation, tumor cells will secret various angiogenic factors such as vascular endothelial growth factor (VEGF) to stimulate rapid development of immature vasculature, which is notoriously tortuous and leaky, leading to significant intravasation of cancer cells. Particularly, vascular density was identified to correlate with the incidence of tumor metastasis[66]. Various nanosystems have exhibited excellent anti-angiogenesis and anti-tumor activities in the past few years[35,36]. Yet, it should also be taken into consideration that anti-angiogenesis may elicit hypoxia, which contributes to tumor aggressiveness and metastasis[67,68]. Such being the case, vasculature normalization rather than inhibition should be regarded as a paradigm in terms of treating metastasis[37]. To achieve this goal, anti-angiogenesis agents should be carefully controlled in dosages or combined with cytotoxic therapeutic approaches like chemotherapy and PDT[38,39].

Additionally, platelets, small bioactive fragments of cytoplasm released from mature megakaryocytes of the bone marrow, also participate in the metastatic cascade[69–72]. Although anti-platelet agents were capable of inhibiting metastasis in animal models, systemically administrating anti-platelet drugs may lead to severe bleeding, which limited the clinical translation[73–75]. Tumor targeted delivery of platelet inhibitors by nano-DDS is a promising strategy to make up for the deficiency. Zhang et al.[40] targeted platelet inhibitor to tumor by tumor-homing peptide (CREKA)-conjugated liposomal nanoparticles (CREKA-Lipo-T). *In vitro*, CREKA-Lipo-T could restrain platelet-tumor adhesion and prevent tumor cells from transitioning into more invasive phenotypes by down-regulating TGF-β. *In vivo*, CREKA-Lipo-T could inhibit outgrowth of metastatic tumor in the highly aggressive 4T1 mouse mammary tumor model. Notably, no bleeding side effort was observed. In a different case, low molecular weight heparin (LMWH) was functioned as hydrophilic segment of micellar nanoparticles to inhibit the adhesion between platelets and cancer cells through blocking P-selectin on activated platelets[41]. The interference of the interaction between platelets and cancer cells suppress EMT and the survival of blood-borne tumor cells.

3.3. *Modulating extracellular matrix*

Extracelluar matrix (ECM) is a sophisticated 3D network composed of fibrous proteins, glycoproteins, enzymes, and matricellular proteins[76]. The ECM of primary tumor plays a pivotal role in metastatic initiation and angiogenesis[77]. During cancer progression, the ECM is continuously remodeled by tumor cells. One of the well-recognized ECM alterations is increased collagen deposition, which regulates cell polarity and migration and is closely related to metastasis[78,79]. Among various components within ECM, matrix metalloproteinases (MMPs), one overexpressed protease that paves ways for tumor migration by directly degrading the barrier, are considered to be an ideal target for metastasis treatment. The past two decades have seen the studies of synthetic inhibitors of MMPs (MMPI) in a variety of cancer types[80]. Marimastat, a broad-spectrum MMPI with poor water solubility, was assembled with hyaluronic acid (HA)-paclitaxel (PTX) prodrug into nanoparticles for metastatic cancer treatment[43]. Significant suppression of tumor growth, lung metastasis and angiogenesis were demonstrated in 4T1 tumor-bearing mice, owing to intracellular MMPs inhibition and cytotoxicity. However, the clinical potential of MMPI is limited, since broad-spectrum MMPI like marimastat may also inhibit some MMPs with antitumor effects such as MMP-3, -8, -11, −12 and −19[81]. Besides, significant fibrosis and musculoskeletal syndrome were observed due to the wide-reaching effects, resulting failure in clinical trials[82]. Whether targeting delivery of MMPIs into tumor cells could solve these problems remains to be answered. Alternatively, repairing the loose and degraded structure of ECM and further restoring the normal cell-ECM adhesion may be a feasible strategy. Laminin is a component of ECM[83]. After binding to integrins or LN receptors of tumor cells, it would self-assemble into fibrils[84]. Inspired by the special property of laminin, a biomimetic device based on laminin-mimic peptide 1 was developed to construct artificial ECM, which could significantly inhibit tumor invasion and metastasis[44] (Fig. 4).

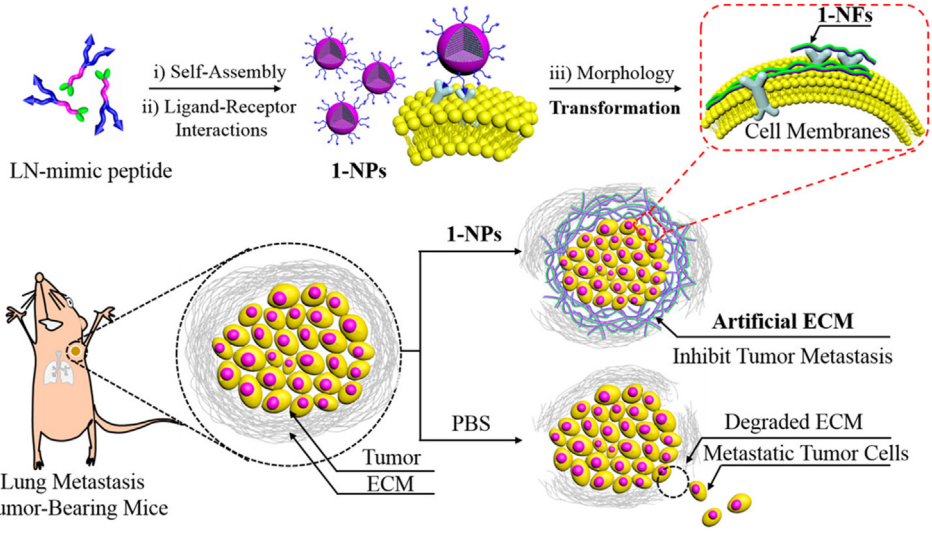

Figure 4 Schematic illustration of the biomimic construction of AECM based on transformable 1-NPs for high-efficient inhibition of tumor invasion and metastasis. Reprinted with permission from Ref. 44. Copyright © 2017 American Chemical Society.

3.4. Targeting tumor stromal cells

Cancer-associated fibroblasts (CAFs), a heterogeneous population of activated fibroblasts within PTME, are involved in multiple pathways that promote tumor progression and metastasis. CAFs exhibit increased secretion of various growth factors and cytokines, such as TGFβ, CXCL12 and IL-6, all of which are shown to enhance invasion and migration of tumor cells[85–90]. As one of the most abundant components of tumor stroma, CAFs are therefore conspicuous targets. Zhao et al.[45] demonstrated that local depletion of CAFs could not only modulate TME, but also enhance the efficacy of chemotherapeutics. Though effective the CAFs depleting strategy may be, it is concomitant with the risk of tumor invasion or migration once tumor cells are not eradicated[91]. To eliminate the potential risks, a CAFs-targeting nanosystem (PNP/siCXCL12/mAb) was devised to delivery siRNA to silence CXCL12, which maintains the activated phenotype of CAFs and participates in tumor progression and metastasis as we demonstrate above[46]. PNP/siCXCL12/mAb was proved to effectively down-regulate CXCL12 by 64.4%, inhibit tumor invasion, migration, and tumor angiogenesis. More importantly, little tumor luminescence was detected in major organs, suggesting metastasis was significantly suppressed.

Macrophages are well-recognized members of immune cells, playing critical roles in immune defense. With the progression of tumors, circulating monocytes infiltrate into tumor tissue, mature and differentiate into multiple subtypes of TAMs, which are revealed to perform protumoral functions including promoting tumor invasion and metastatic dissemination[92–94]. Notably, the functions of macrophages depend on their phenotypes. M1 macrophages involve in a series of immune modulation that suppress tumor progression while M2 macrophages support tumor progression and evasion from immune surveillance. Various tactics based on nanomedicine are shown to effectively modulate TAMs by selectively killing M2 macrophages or reeducating M2 macrophages to M1 macrophages. Zang et al.[50] developed zoledronate-loaded lipid-coated calcium nanoparticles (CaZol@pMNPs) conjugated with mannose, which targets the CD206 of M2-like macrophages. The targeted delivery of zoledronate to TAMs enables their specific apoptosis, thus reversing immunosuppressive microenvironment and suppressing tumor growth. Besides, a safe genetic reprogramming strategy was proposed by Zhang et al.[51], who delivered *in vitro*-transcribed mRNA encoding M1-polarizing transcription factors *via* polymeric nanoparticles to repolarize TAMs. After intravenously injecting, the nanosystem showed impressive anti-metastasis efficacy without disrupting immune homeostasis.

3.5. Targeting EMT

The epithelial mesenchymal transition (EMT) is defined as a shift from epithelial state to mesenchymal state, facilitating tumor cells to approach the vessels and get into circulation. Specifically, tumor cells at the frontline of invasive tumors usually exhibit a loss of epithelial markers and intercellular adhesions and an increase of the expression of mesenchymal markers[95]. Gradually, tumor cells transform into mesenchymal phenotypes, which are characterized by enhanced motility and contractility, coupled with increased expression of metastasis-associated molecules like MMPs and VEGFs[96]. EMT is demonstrated to be induced by a wide range of agents including WNT, HIF-1α, TGF-β and even cytotoxic drugs[61,97–100]. Intervening EMT may serve a purpose to inhibit the initiation of metastasis, so Huang et al.[53] constructed ZnAs@SiO2 nanoparticles, which could promote SHP-1 while inactivate JAK2/STAT3 pathways, thus regulating the underlying gene networks and coinstantaneously inhibiting stemness and EMT. As we discussed above, certain chemotherapeutics like DOX and paclitaxel are able to trigger EMT, increasing the risks of metastasis. To eliminate these side effects, Zhou et al.[54] co-delivered DOX and a TGF-β receptor inhibitor *via* hydroxyethyl starch-polylactide (HES-PLA) nanoparticles to suppress primary tumor and simultaneously inhibit pulmonary metastasis. Although heterogeneous distribution of DOX could exacerbate EMT and metastasis, the co-delivered TGF-β receptor inhibitor was able to silence the activated TGF-β pathway, thereby suppressing the EMT process. In addition to regulate EMT-associated signal pathways, a different tactic focusing on depleting EMT-type cancer cells was also proved to effectively weaken the metastatic potential of primary tumor[56].

Besides EMT, mesenchymal to epithelial transition (MET), which is the reversion of EMT, also participates in metastatic cascades[101]. For the disseminated cancer cells, MET may facilitate metastatic colonization[102]. As a result, therapeutic agents that inhibit EMT may promote the CTCs colonization of distant organs. Moreover, by the time tumor masses are detected, a large number of tumor cells may have already detached from primary soil and spread far away. Hence, targeting EMT alone may be counterproductive to achieve anti-metastasis effect. Treatment options that suppress both EMT and MET or directly targeting mesenchymal tumor cells are theoretically more effective.

3.6. Targeting the cancer stem cells (CSCs)

CSCs are notoriously a kind of multipotent cells with the ability to self-renew and initiate new tumors. Acquisition of stem-like properties is usually concurrent with the transition to an invasive mesenchymal phenotype with higher metastatic capability[103]. Indeed, during metastatic dissemination, only a small number of circulating tumor cells (CTCs) survive to form micrometastasis, this subpopulation is considered to be CSC phenotype[104,105]. In other words, CSCs can be regarded as the seeds that hold the best chance to survive and colonize the secondary soil. Thus, targeting and further eliminating CSCs is anticipated to not only eradicate primary tumors but prevent metastasis. For instance, Li et al.[58] designed integrin α5 -targeting nanoparticles to specifically inhibit the Wnt/β-catenin pathway, which is critical in the generation and maintenance of stem cells. Upon systematic administration, nanoparticles showed enhanced accumulation and retention, which helped to effectively down-regulate β-catenin levels and inhibit both primary and metastatic tumors. Notably, certain chemotherapeutics were illustrated to promote stemness and metastasis *via* a "backdoor" effect mediated by cyclooxygenase-2/prostaglandin-E2 (COX-2/PGE2) signaling[106]. To block the "backdoor", a specific COX-2 inhibitor was co-delivered with DOX to enhance antitumor activity while eliminate DOX-induced proliferation of cancer stem-like cells[59]. The combined treatment not only efficiently inhibited primary tumor growth by 91%, but also suppressed pulmonary metastasis by 67%. Collectively, these encouraging results implicate that developing nanocarriers to enhance efficiency of CSCs targeting therapy is a promising approach to prevent the soil-to-seed process and subsequent metastatic dissemination.

4. Targeting the circulating tumor cells (seeds) through engineering nanosystems: Stifling metastasis in the half-way

As mentioned, tumor cells that have undergone EMT are usually endowed with mesenchymal features, stem-like properties and enhanced migration ability, which allow them to infiltrate into circulation and disseminate. This kind of cells is termed as circulating cancer cells (CTCs). Once entering vasculature, CTCs are faced with a variety of life-threatening agents, including physical pressure, oxidative stress and innate immune cells[107]. Even though they can adhere to platelets or form CTC clusters to protect themselves from damages,[108,109] only a small number of CTCs will survive to extravasate and seed in distant organs[110]. According to the "seed and soil" theory, CTCs are exactly the seeds that detach from primary soil with potential to seed in the second soil[111]. Moreover, CTCs are inversely associated with the survival rates of patients, and always considered as important indicators that provide valuable information for diagnosis and prognosis of metastasis[112]. Due to the pivotal role of CTCs in the metastasis, targeting therapeutic agents to CTCs to deplete them is anticipated to stifle the metastatic cascade in the half-way. Unlike targeting cancer cells at the tumor site, CTC-targeting nanoparticles are required to have a particularly long circulation time and high affinity towards CTCs with least drug leakage since CTCs are extremely rare in peripheral blood, approximately one out of a billion hematological cells[113,114]. Besides, though CTCs are identified to express both EMT and CSCs markers[115], they may obtain extra molecular changes during EMT and circulation. Specifically, multiple cellular mechanisms are activated and CTCs are modified to be heterogeneous with enhanced metastatic potential, bringing extra challenges for targeted drug delivery[116,117].

Fortunately, various novel targeting tools as well as nanovehicles have been introduced to broaden the arsenal fighting against CTCs by virtue of advanced molecular and biomedical engineering technology. These nanosystems showed excellent CTC-targeting ability (Table 2[41,118-126]).

Among various versatile nanomedicine-based DDS, biomimetic nanoplatforms, which are capable of simultaneously evading immune systems, crossing physical barriers and maintaining various surface biomarkers, hold great chance to address the predicament[127]. Notably, the camouflage of cell membrane can extend the systemic circulation of encapsulated nanoparticles. For example, owing to the immune evasion characteristic, the elimination half-life of red blood cells (RBC) membrane coated nanoparticles was extended to 39.6 h while that of PEG-coated nanoparticles was 15.8 h[128]. Besides, RBC membrane endowed nanoparticles with better stability in phosphate buffered solution and serum[129]. Therefore, biomimetic strategies provide ideal platforms for targeted capture and elimination of CTCs. Inspired by CTC's platelet-dependent evasion of host immune surveillance, Ye et al.[121] devised platelet membranes coated nanoparticles, named nanoplatelets, loaded with indocyanine green as well as DOX to track and eliminate CTCs in vasculature (Fig. 5). *In vivo* studies, owing to the high affinity between platelets membrane-presented P-selectin and CD44 of tumor cells, mice pre-treated with nanoplatelets withstood the challenge of intravenous injection of cancer cells, exhibiting significant decreased pulmonary metastasis. Moreover, nanoplatelets could efficiently penetrate into deeper lymph nodes through lymphatic circulation after intratumoral administration. After further irradiation with 808 nm NIR light, lymph node and primary tumor were efficiently reduced. Interestingly, both hepatic and pulmonary metastases

Table 2 Summary of emerging anti-metastasis strategies based on targeted elimination of CTCs.

Nanocarriers	Modification	Payload	Strategy	Ref.
Magnetic nanocore with multiple iron oxide nanoparticles	Gold nanocage satellites and anti-epithelial cell adhesion molecule	None	Isolate CTCs by magnetic enrichment and eradicate CTCs	118
Dextran-octadecanoic acid micelles	Sialic acid	Doxorubicin	E-selectin mediated targeted clearance	119
Peptide-based nanoparticle	Cyclic RGD peptide and pH-sensitive polyhistidine sequence	siRNA	Down-regulate TF expression and prevent platelet adhesion around CTCs	120
PLGA nanoparticle	Platelet membrane coating	Doxorubicin and indocyanine green	Capture and destroy CTCs	121
LMWH-TOS micellar nanoparticle	Phenylboronic acid	Doxorubicin	Inhibit the interactions between tumor cells and platelets	41
Dendrimer G4.5	Two double strand circular aptamers	None	Apoptosize CTCs and inhibit their bioenergetic activities	122
Mesoporous silica nanoparticle	Two aptamers	Doxorubicin	Inhibit CTCs viability and the adhesion of cancer cells to the endothelium and the consequent transmembrane migration	123
Polymer nanoparticle	K237 peptide and Ep23 aptamer	Paclitaxel	Capture and neutralize CTCs in bloodstream	124
DNA tetrahedron	Hairpin switch aptamer	Doxorubicin and photosensitizer	Destroy CTCs	125
Nanoparticle	CD44v6-peptide	CdTe quantum dots	Bind to CD44v6 expressing tumor cells, block the function of CD44v6 protein and visualize CTCs	126

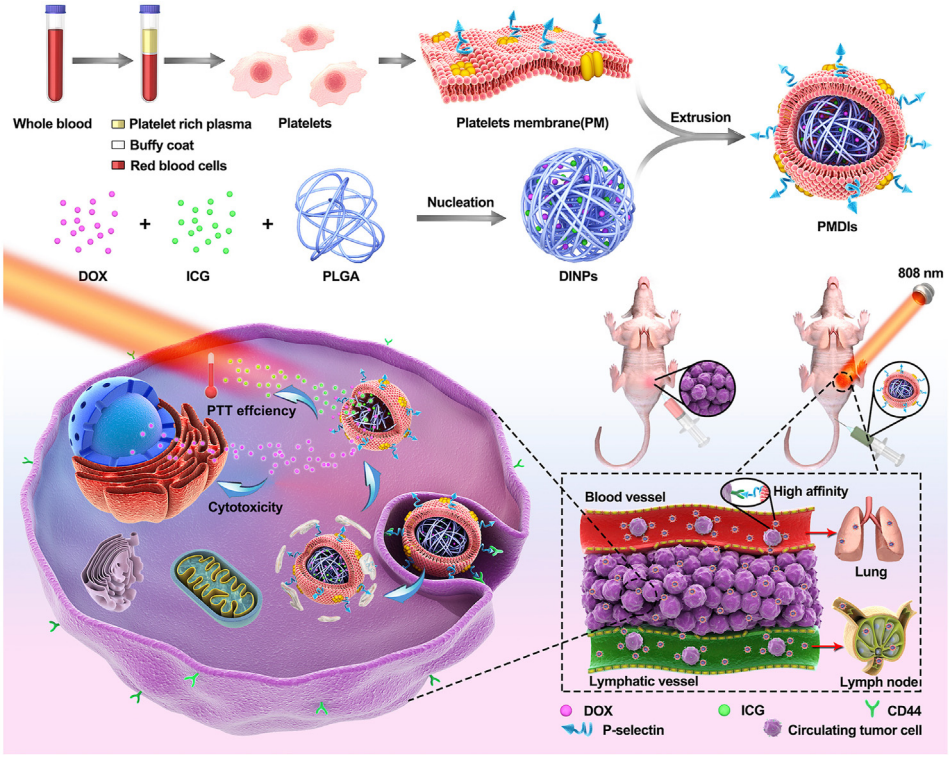

Figure 5 Preparation process of nanoplatelets and their synergistic effects against breast cancer metastasis by active targeting of circulating tumor cells and combination treatment of chemotherapy and photothermal therapy. Reprinted with permission from Ref. 121. Copyright © 2019 Elsevier.

were also inhibited, suggesting that nanoplatelets might also be able to hijack CTCs in lymphatic circulation. Also, neutrophils were proved to interact with CTCs and promote their extravasation process[130]. With this point in mind, Kang et al.[131] designed another biomimetic drug delivery system by coating nanoparticles with neutrophils membranes (NM-NPs), which reserved various adhesion molecules for CTCs targeting. By virtue of CTCs depletion, both early metastasis initiation and established metastasis were suppressed. Besides biomimetic approaches, a different strategy that specific delivering siRNA to down-regulate tumor-associated tissue factor (TF) in CTCs were corroborated to hinder the platelets adhesion around CTCs, thus decreasing the survival rate of CTCs[120]. Another platelets-CTCs interaction blocking strategy was proposed by our group[41], wherein low molecular weight heparin served as hydrophilic segment of micellar nanoparticles to hinder P-selectin on activated platelets. As a result, the platelet coats of CTCs were taken off and CTCs died of immune clearance or shear pressure[41]. It is worth noting that CTCs are elicited to express different biomarkers during their release and dissemination, so mono-targeting approaches may be insufficient to eliminate this heterogeneous group of tumor cells. On the contrary, nanoparticles with multiple target heads may be more powerful for CTCs depletion[122–124].

5. Modulating premetastatic niche (premetastatic soil) *via* nano-DDS: Inhibiting metastatic colonization

Despite the great metastatic potential, CTCs alone are still not enough to form metastasis. To successfully sow the surviving CTCs into the secondary soil, the primary soil will secrete tumor-derived factors and extracellular vesicles (EVs) prior to the dissemination of tumor cells to reshape the potential metastatic sites into favorable environment[132,133], which is termed as pre-metastatic niche (PMN). In detail, the PMN will undergo a series of alternations including increased adhesion molecules on the endothelial cells, enhanced vascular permeability, recruitment of myeloid cells, overexpressed inflammatory molecules, up-regulated MMP-9 in the ECM and hypoxia[133–138]. These factors synergize to construct immunosuppressive and inflammatory microenvironment, facilitating extravasation, invasion, and colonization of CTCs. Targeting PMN and further rendering it less hospitable for CTCs is an ideal protocol to inhibit the last several steps of the metastatic cascade.

Nonetheless, only a few strategies targeting PMN have been introduced since many molecular and cellular mechanisms remain to be illustrated. Besides, owing to the similar physiological nature between PMN and normal tissues, it may sounds challenging to specifically target therapeutic agents to PMN without extra influence to normal tissues. Actually, some specific organs such as lungs are pre-conditioned by primary tumors and are endowed with hyper-permeability, which may benefit nanoparticles of appropriate sizes[139–141]. Indeed, it was corroborated that liposomes of 100 nm showed significantly enhanced accumulation in lungs at early stages of metastatic progression, even before metastasis are visualized by MRI[142]. Current reports on modulating PMN have shown encouraging therapeutic outcomes, indicating that PMN can be regarded as a promising target of tumor metastasis (Table 3[131,143-148]).

Table 3 Summary of emerging nano-DDS based therapeutic approaches for modulating premetastatic niche.

Vehicle	Modification	Therapeutics	Advantage	Ref.
Poly (latic-*co*-glycolic acid) nanoparticle	Neutrophils membrane coating	Carfilzomib	Down-regulate the expression levels of S100A9 and stromal cell-derived cytokines	131
Micelle	Fucoidan coating	Metformin and DHA	Inhibit CTCs adhesion to activated endothelial cells, alleviate lung vascular permeability and reverse the aberrant expression of fibronectin MMP-9, and S100A9	143
Hydroxyapatite nanoparticle	Cathepsin B enzyme-sensitive peptides AL-linked peptide module combination (PMC) shell consisting of CREKA and KLA	Doxorubicin	Block the mitochondrial escape signaling pathways	144
Positively charged mesoporous silica nanoparticle	EGFR-targeting aptamers	The binding effect of the carrier itself	Bind blood-borne negatively charged oncogenic exosomes and tow them into the small intestine	145
Gold nanocage	Platelet and neutrophil hybrid cell membrane coating	Doxorubicin and indocyanine green	Neutralize tumor-derived exosomes and enhance immune microenvironment	146
LMWH-TOS micelle	Phenylboronic acid	Doxorubicin and immunopotentiator (α-galactosylceramide)	Inhibit early pulmonary recruitment of granulocytic myeloid-derived suppressor cells and down-regulate their expression of MMP-9	147
Biomimetic nanoparticle	Autologous breast cancer cells derived exosome membrane coating	Cationic bovine serum albumin conjugated siS100A4	Down-regulate the expression of S100A4	148

As we pointed out before, primary tumor-derived exosomes are one of the most important culprits of the formulation of PMN, binding to immune cells to inhibit their normal function. Unfortunately, this binding activity cannot be blocked by monoclonal antibodies[149]. Ye et al.[146] developed a novel nanosystem coated with platelet and neutrophil hybrid cell membrane, which functioned to capture and eliminate tumor-derived exosomes, thereby reversing immunosuppressive PMN. Similarly, a smart nanobiomaterial was constructed, wherein positively charged mesoporous silica nanoparticles were functionalized with EGFR-targeting aptamers to specifically recognize and bind negatively charged oncogenic exosomes. Next, exosomes were towed across hepatobiliary layers and Oddi's sphincter into the small intestine. Consequently, the blood-borne exosomes were eliminated, the initiation of PMN formulation was stopped and pulmonary metastasis was attenuated in mice[145] (Fig. 6). Inflammation, another crucial characteristic of PMN, can be activated by S100 proteins, inflammatory cytokines and various signal pathways such as nuclear factor-κB (NF-κB) and signal transducer and activator of transcription-3 (STAT3)[150]. To release the inflammatory microenvironment and further modulate PMN, Jiang et al.[143] synergized metformin and docosahexaenoic acid (DHA), two anti-inflammatory agents, to inhibit multiple inflammatory pathways. In order to synthesize the co-delivery system, a metformin derivative (OA−Met) was designed as amphiphilic agent to form micelles in water and hydrophobic agent DHA was entrapped into the hydrophobic cores of micelles. Furthermore, fucoidan was utilized as coating material due to its high affinity to the over-expressed P-selectin on endothelial cells of PMN. In the subsequent experiments, the novel micelles were certified to modulate PMN by inhibiting CTCs adhesion to endothelial cells, alleviating vascular permeability and normalizing aberrant expression of specific proteins such as S100A9 and MMP-9, thereby preventing the formation of lung metastasis. When combined with chemotherapy, this nanosystem could suppress both primary tumor and metastasis in an orthotopic breast tumor mice model. Inspired by these positive results, our group[147] established micellar nanoparticles to inhibit pulmonary recruitment of granulocytic myeloid-derived suppressor cells (G-MDSCs), which are responsible for vascular leakiness and immunosuppressive PMN of melanoma and mammary cancers. In this micellar nanosystem, low molecular weight heparin served as hydrophilic segment to interrupt the P-selectin-mediated adhesion between G-MDSCs and endothelial cells while D-α-tocopheryl succinate (TOS) served as hydrophobic segment to down-regulate MMP-9 in G-MDSCs (Fig. 7). According to the dextran permeability assay, the vessel abnormalities in premetastatic lungs were effectively prevented, indicating that this nanosystem could also block the interaction between seeds and soil. Moreover, after loaded with DOX and immunopotentiator, this nanoplatform could simultaneously achieve long time inhibition of relapsed melanoma and postoperative metastasis. Apart from this, a different strategy focused on an upstream target, S100A9, which is responsible for the recruitment of dierent subsets of myeloid cells to PMN. With the coating of neutrophil membranes, nanoparticles were endowed with PMN homing property, enabling them to accumulate in premetastatic lungs and downregulate S100A9 as well as stromal cell-derived cytokines, including MMP2, CXCL12 and TNF-α[131]. Another biomimetic strategy utilized the organ-tropic nature of exosomes to target PMN[148]. By coating with breast cancer cells-

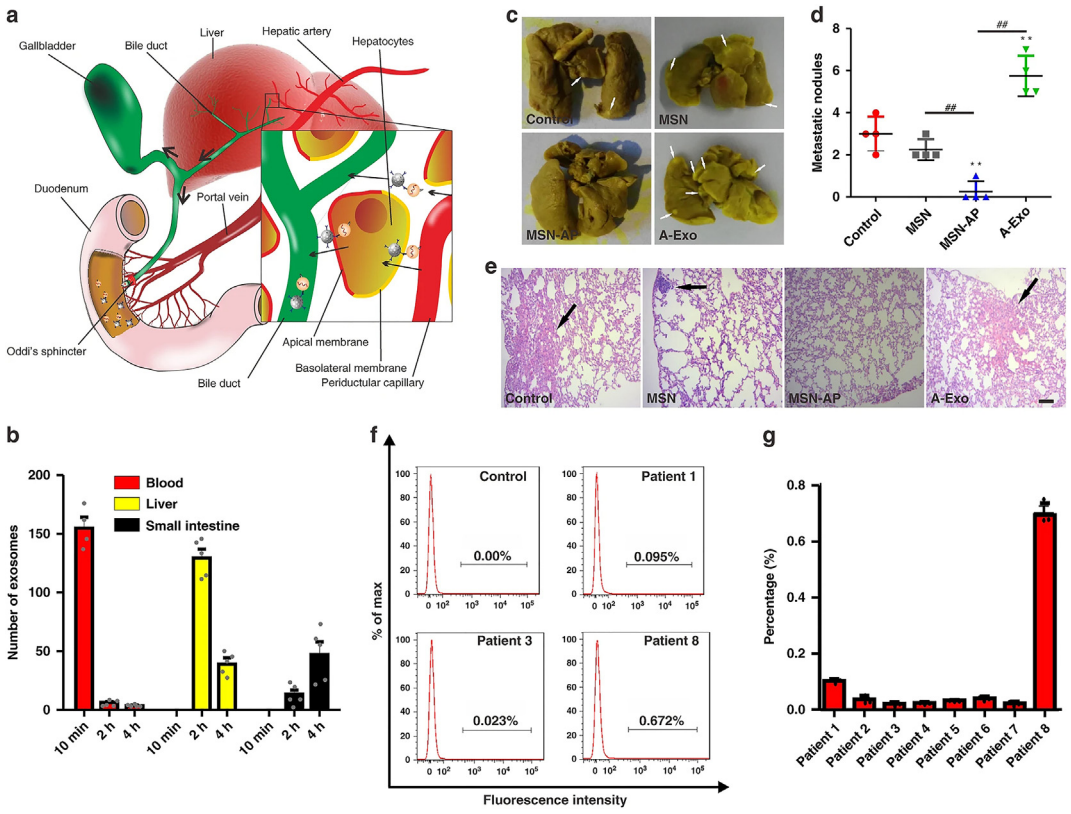

Figure 6 Elimination or deactivation of circulating exosomes by MSN-AP in animals and patient blood. (a) Schematic showing that MSN-AP binds to and tows circulating exosomes in the liver into the space of Disse, and the conjugated MSN-Exo can be endocytosed by polarised hepatocytes, transcytosed through the hepatocytes and enter the bile duct and small intestines *via* the sphincter of Oddi. (b) Dynamic decrease in blood A-Exo was sequentially accompanied by an increase of A-Exo in the small intestines of mice when MSN-AP was intravenously administered. $n = 5$. (c) Photos and (d) quantification of lung metastatic nodules developed (arrows) following subcutaneous implantation of A549 cells in nude mice receiving intravenous A-Exo (2 μg), saline, MSN or MSN-AP (both 5 mg per kg) every 3 days starting on day 14 after A549 implantation for an additional 3 weeks. $n = 4$ mice. (e) Lung H&E stains to show tumours. Scale bars = 200 μm. (f) Flow cytometry analysis and g quantification of patient EGFR-exosomes captured by MSN-AP. Note that patient 8 was in a late stage of lung cancer. $n = 8$ patients. Data presented as the mean ± SEM. **, ## $P < 0.01$; one-way ANOVA (d). The *Y*-axis of Fig. 6b represents the average number of five randomly selected single fields of vision. Source data are provided as a Source Data file. Reprinted with permission from Ref. 145. Copyright © 2019 Springer Nature.

derived exosome membranes, Zhao et al.[148] delivered siRNA to silence pulmonary S100A4, which contributed to formation of PMN and tumor progression. The biodistribution data indicated higher accumulation of exosome membrane-coated nanoparticles due to the surface integrins that co-locate in the laminin-rich lung microenvironment. As a result, this biomimetic approach showed effective inhibition of postoperative metastasis in triple negative breast cancer. Together, modulating PMN is an effective tactic to prevent the disseminated seeds from taking root in the secondary soil, eventually resulting in CTCs death in the circulation. With more efforts dedicated in the biology and targeting mechanisms, PMN may become a hotspot for metastasis treatment.

6. Multiphase-targeted inhibition of metastatic cascade by nano-DDS

As we discussed in the second part of this review article, tumor metastasis means sophisticated crosstalk among primary soil, seeds and secondary soil. Each phase of metastasis is usually composed of numerous pathways and biological events. Therefore, it is not realistic to rely on monotherapy to completely cut off the metastatic cascade, since there are many alternative routes for cancer cells to successfully metastasize. For instance, simply by targeting the primary soil, the risks of metastasis cannot be totally eliminated and the supportive secondary soil is always ready to welcome the colonization of survived CTCs. Also, though blood-borne CTCs are captured and killed by therapeutic approaches, the primary tumor cells could still metastasize through lymphatic system. Alternatively, targeting multiphase metastasis rather than focusing on a single stage may be a more reliable strategy, which stands a better chance to terminate the metastatic cascade. Nanotechnology-based DDS presents a promising platform to realize this goal, either by the multifunctional drug carrier materials, or the co-delivery nanovehicles, which are capable of incorporating many anti-cancer and anti-metastasis agents into one system to achieve synergistic effects or target multiple pathways. The novel nano-DDS makes it possible to simultaneously target primary soil for the inhibition of metastatic initiation, CTCs for suspension of metastatic dissemination and secondary soil for inhibition of metastatic colonization (Table 4[41,119,120,123,124,131,146,147]).

For example, our group[41,147,151] developed a novel multifunctional anti-metastasis micellar nanovehicle, which can be

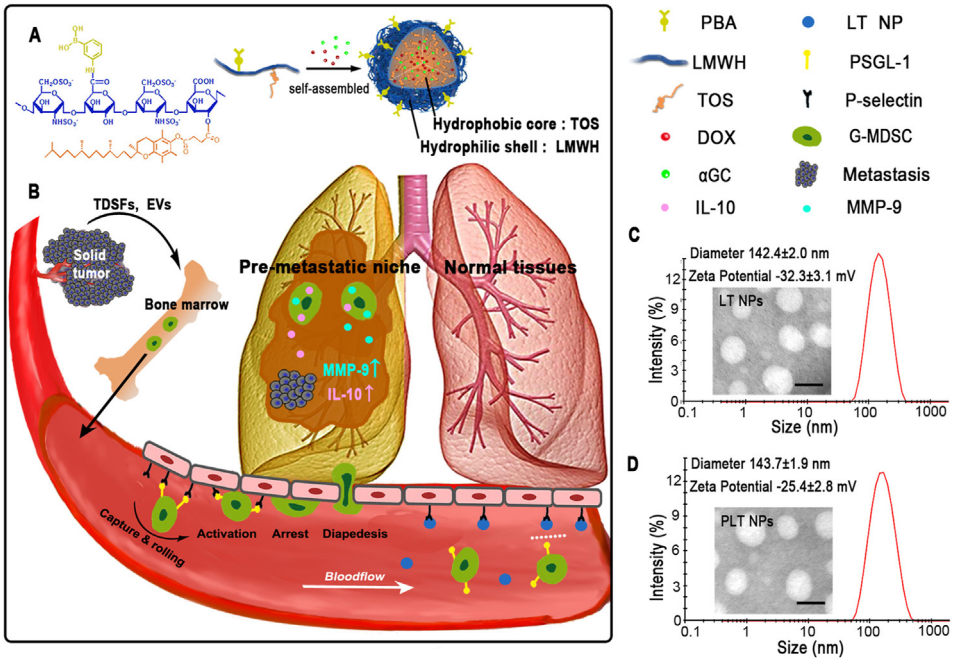

Figure 7 (A) Schematic illustration of PLT/DOX/αGC NPs (B) Schematic illustration of the anti-G-MDSC recruitment mechanism of NPs (C) Dynamic light scattering (DLS) size distribution and transmission electron microscopy (TEM) image of LT NPs. The scale bar represents 150 nm (D) Dynamic light scattering (DLS) size distribution and transmission electron microscopy (TEM) image of PLT NPs. The scale bar represents 150 nm. Reprinted with permission from Ref. 147. Copyright © 2020 American Chemical Society.

regarded as a paradigm of multiphase-targeted inhibition of metastatic cascade. The micellar nanovehicle consists of three parts: low molecular heparin (LMWH) as the hydrophilic segment, D-α-tocopheryl succinate (TOS) as the hydrophobic segment, and phenylboronic acid (PBA) targeting the sialic acid (SA) residues on tumor cells[41,147,151]. Independent of its anti-coagulant activity, LMWH has many other pharmacological properties, including but not limited to anti-tumor and anti-metastasis[152]. The anti-metastasis activity of LMWH can be attributed to its ability to bind to growth factors and adhesion

Table 4 Summary of current multiphase-targeted anti-metastasis nanosystems.

Design	Strategy	Target	Ref
K237 peptide and Ep23 aptamer dual functionalized paclitaxel-loaded polymer nanoparticle	Target neovasculature and eradicate CTCs	Metastatic initiation and dissemination	124
PBA-LMWH-TOS micellar nanoparticle loaded with doxorubicin	Inhibit the expression of MMP-9 in tumor cells	Metastatic initiation and dissemination	41
	Cut off tumor cell−platelets interactions		41
Peptide-based self-assembling TF siRNA delivery system	Knock down TF expression in both TME and CTCs	Metastatic initiation and dissemination	120
	Cut off tumor cell−platelets interactions		120
	Reverse the hypercoagulable state of TME		120
Neutrophils membrane coated PLGA nanoparticle loaded with carfilzomib	Deplete CTCs in circulation and inhibit the formulation of PMN	Metastatic dissemination and colonization	131
E-selectin-targeting doxorubicin-loaded sialic acid-dextran-octadecanoic acid micelles	Inhibit cell migration	Metastatic initiation and dissemination	119
	Kill blood-borne CTCs		119
	Shrink established lesions		119
EpCAM and CD44 dual targeting mesoporous silica nanoparticle loaded with doxorubicin	Eliminate CTCs	Metastatic dissemination and colonization	123
	Inhibit extravasation of CTCs		123
Platelet and neutrophil hybrid cell membrane coated nanocage loaded with doxorubicin and indocyanine green	Capture and clear CTCs and tumor derived exosomes	Metastatic initiation, dissemination and colonization	146
	Reverse the immunosuppressive TME		146
PBA-LMWH-TOS nanoparticle loaded with an immunopotentiator and doxorubicin	Inhibit CTCs implantation	Metastatic dissemination and colonization	147
	Interfere the PMN-tropic recruitment and vascular destruction of G-MDSCs		147
	Down-regulate MMP-9 expression of G-MDSCs		147

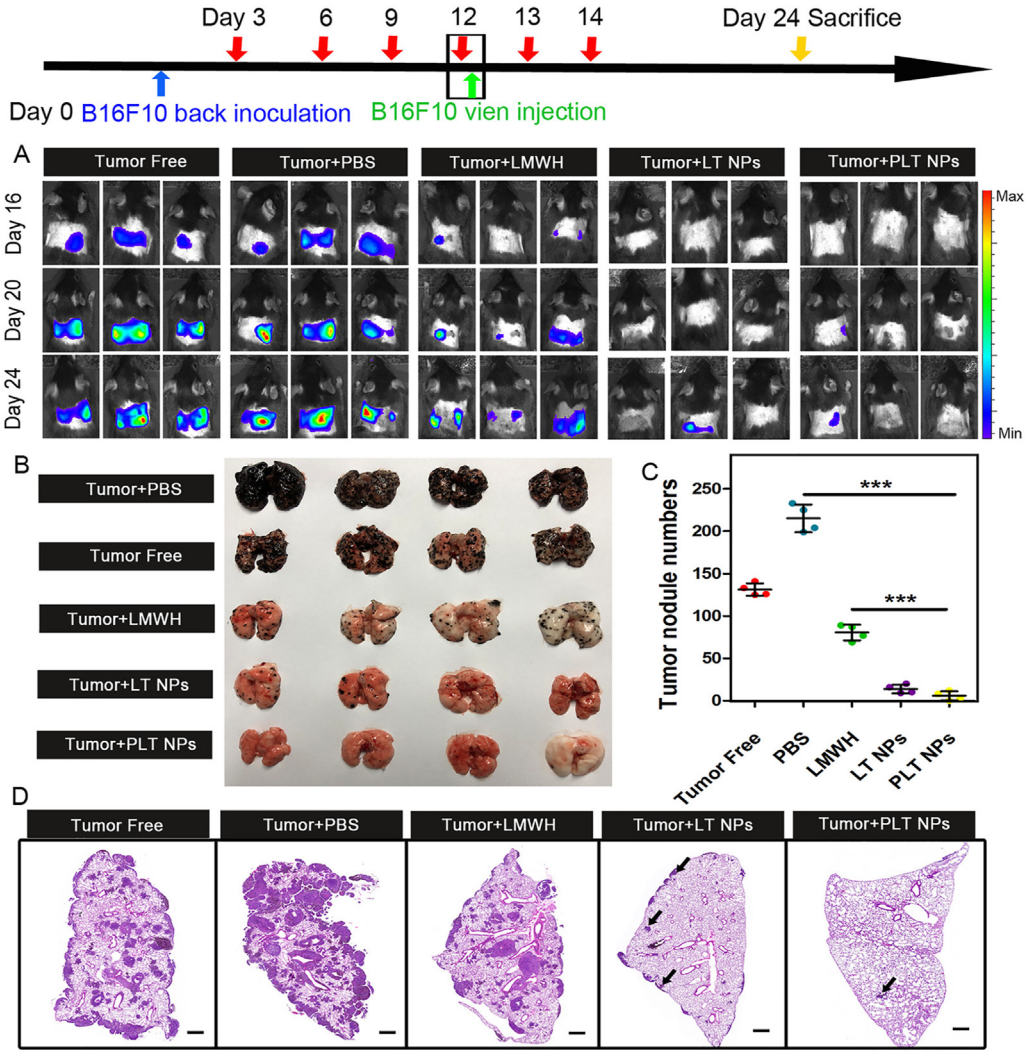

Figure 8 Antimetastatic treatment *in vivo* (A) Representative *in vivo* fluorescence images of the lungs of mice treated with PBS, LMWH, LT NPs, and PLT NPs and tumor-free lungs, $n = 4$ (B) Images of harvested lungs (C) number of B16F10 metastatic nodules on the lungs, and (D) representative images from the histological analysis (H&E assays) of the lungs of B16F10 metastasis model mice after treatment with PBS, free LMWH, LT NPs, or PLT NPs and the lungs of tumor-free mice (means ± SD, $n = 4$, $^{***}P < 0.001$). The dark purple parts indicated by arrows are metastatic nodules. The scale bar represents 500 μm. Reprinted with permission from Ref. 147. Copyright © 2020 American Chemical Society.

proteins, among which selectin is a key mediator of tumor cell-platelet and tumor cell-endothelial cell interactions[153–155]. Indeed, we corroborated that LMWH (hydrophilic segment) could effectively inhibit P-selectin-mediated adhesion between tumor cells and platelets as well as vascular endothelial cells and G-MDSCs. As a result, CTCs in the blood flow lost the shield of platelets and died of shear pressure or immune clearance. Besides, the extravasation of G-MDSCs towards PMN was significantly suppressed and the formulation of PMN was prevented. The hydrophobic segment, TOS, also served as an anti-metastasis agent to inhibit the expression of MMP-9 in both G-MDSCs and B16F10 cells. Hence, the structure of ECM of both primary and secondary soil was reinforced, thereby restraining the invasion and colonization of tumor cells. The subsequent evaluation showed that even the blank nanoparticles exhibited significant anti-metastasis ability (Fig. 8). In a different case, a neutrophil-mimicking DDS was constructed, wherein the neutrophils membrane-associated protein cocktails were preserved to maintain the binding ability[131]. The surface-anchored protein cocktails including LFA-1, L-selectin and $\beta 1$ integrin enabled the nanosystem specifically recognize CTCs and inflamed endothelial cells of PMN, thereby achieving dual targeting of seeds and secondary soil (Fig. 9). After being loaded with a proteasome inhibitor, carfilzomib, the nanosystem facilitated CTCs apoptosis in circulation selectively, prevented early metastasis and suppressed the progression of established metastasis. Likewise, inspired by the PMN-tropic and CTCs-adherent nature of neutrophils and platelets, Ye et al.[146] developed platelet and neutrophil hybrid cell membrane-coated gold nanocages called nanosponges. The nanosponges were further loaded with doxorubicin (DOX) and indocyanine green (ICG) to actively clear blood-borne CTCs as well as tumor-derived exosomes, which account for immunosuppressive microenvironment. As a result, both liver and lung cancer metastasis were inhibited, which may

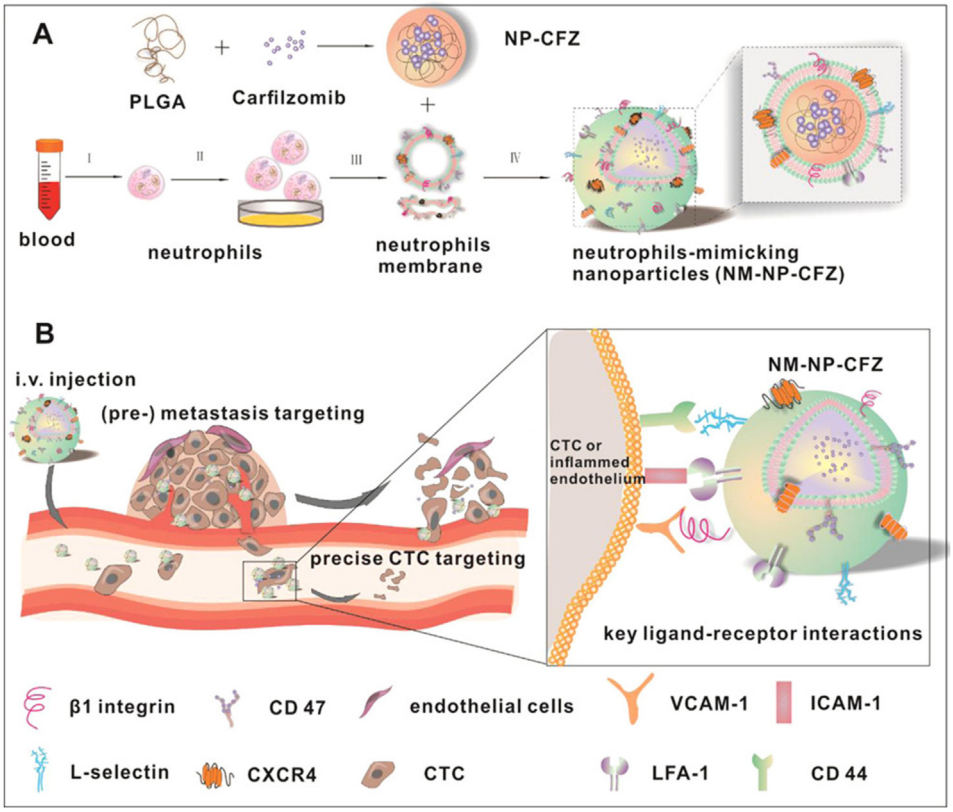

Figure 9 (A) Protocol for the synthesis of NM-NP-CFZ (I) Neutrophils extraction from the whole blood using Percoll gradient separation method (II) Lipopolysaccharide (LPS) stimulation of the isolated neutrophils (III) Plasma membrane of the LPS-stimulated neutrophils isolated by centrifugation (IV) NM-NP-CFZ was synthesized by coating the plasma membrane of the LPS-stimulated neutrophils on the poly (latic-*co*-glycolic acid) (PLGA) NPs. (B) The cocktail of neutrophils membrane-associated proteins enables the resulting NM-NP-CFZ to target CTCs in circulation and inflamed endothelial cells in the premetastatic lesion. Three pairs of key interactions including the binding of LFA-1 with ICAM-1, CD44 with L-selectin, and β1 integrin with VCAM-1 were involved in the CTC- and inflamed endothelium-targeting of NM-NPs. Reprinted with permission from Ref. 131. Copyright © 2017 American Chemical Society.

owe to the enhanced immune microenvironment of primary tumor and PMN as well as suppressed metastatic dissemination.

7. Targeting established metastasis with nano-DDS

For quite a few patients, tumor cells may have already spread systemically or even colonized by the time they are diagnosed. Therefore, strategies mentioned above can only prevent or restrain the progression of established metastasis, but not enough to wipe out them. To treat established metastasis, therapeutic agents are required to kill tumor cells rather than just keep cytostatic. However, some biological characteristics of metastatic tumors may bring tricky difficulties for drug delivery. During the metastatic cascade, the disseminated tumor cells gain numerous molecular alternations through epigenetic and genetic changes[156]. As a result, different levels of biomarkers are observed between primary and metastatic tumors[157,158]. So nanoparticles that target receptors on primary tumors may not be able to apply to metastatic tumors. Within metastatic foci, there also exists intratumoral heterogeneity, which additionally poses challenges for therapeutic elimination[159]. Besides, the well-known EPR effect is absent in the metastatic tumor due to the immature neo-vascular, thereby significantly weakening the permeability of nanoparticles. Thus, it is challenging to devise effective carriers to enhance the penetration of payloads and eliminate metastasis.

As tumor cells spread throughout the body, they will find a suitable site for colonization. Among various organs, lymph nodes, lungs, brain, bone and liver are preferred choices[160]. Some organs may bring extra burden for targeting delivery due to their natural features. A typical case is central nervous system (CNS) metastasis, which refuses many therapeutic agents because of the existence of blood−brain barrier (BBB). Engineering nanoparticles have shown impressive ability to transport payloads across BBB[161]. Wen et al.[162] designed CXCL13-modified nanocapsule to encapsulate rituximab (RTX), an anti-cancer antibody suffering from low levels within CNS. The acetylcholine analogues of the polymer shell significantly enhanced the BBB permeability of RTX, increasing the concentration of RTX in CNS by a factor of 10 and thus eliminating lymphomas of brain. Beyond brain metastasis, targeting lymphatic metastasis is also a tricky issue due to the anatomical structure of lymphatic system. Nanoparticles with smaller sizes may benefit from this characteristic[163]. Liu et al.[164] developed clustered nanoparticles with tunable sizes that response to acidity. The cluster nanoparticles have an initial size of ~100 nm, which is in favor of long circulation. Once entering the tumor site, the sizes will change to ~5 nm, facilitating their penetration into solid tumor, diffusing in the interstitial fluid and further intravasate into tumor lymphatics.

Most nanotherapeutic protocols are not powerful enough and easy to lead cancer recurrence, resulting in failure of anti-

metastasis. With the emergence of new weapons such as gene therapy, immunotherapy and photothermal therapy, combination of these therapeutic approaches *via* nanoparticles has showed better overall efficacy and complementary advantages[165]. Photothermal therapy (PTT) is a promising weapon for ablation of local tumors. Nonetheless, its efficiency is seriously weakened in treating metastatic tumors due to poor light penetration, insufficient to eradicate stubborn tumor mass. Nam et al.[166] constructed polydopamine-coated spiky gold nanoparticles as photothermally stable photosensitizers, wherein the polydopamine coating protected the nano-spike structures from photothermal deformation to improve photothermal efficiency. After combined with a subtherapeutic dose of doxorubicin, even a single round of PTT could trigger robust immune responses in virtue of antigens and pro-inflammatory cytokines released by dying tumor cells. This chemo-photothermal therapy eradicated not only local tumors, but also distant tumors in >85% of animals bearing CT26 colon carcinoma. In another case, photodynamic therapy (PDT) and immunotherapy were combined together by integrating photosensitizer and immune checkpoint inhibitor into a chimeric peptide which further self-assembled into nanoparticles[167]. The two therapeutics were complementary within the nanosystem: the PDT elicited tumor apoptosis and release of antigen, thus triggering immune response, which further recruited $CD8^+$ T cells to address the limitation of light. Finally, both primary and lung metastatic tumors were eradicated.

8. Conclusions and future perspectives

In summary, tumor metastasis has caused wide concern due to its poor survival rates and therapeutic outcomes. Existing treatments including surgery, chemotherapy and radiotherapy are far from optimal to effectively address this dilemma. Moreover, several studies have pointed out that surgery and chemotherapeutics may be able to increase the risks of metastatic dissemination. Although anti-metastasis treatments are faced with great challenges, encouraging progress have been made in fighting metastasis through nanotechnology-based DDS. Due to their novel characteristics, nanoparticles can not only improve the properties of payloads, but also mitigate the side effects by minimizing the drug exposure to the normal tissues. In this review article, various nanosystems that interrupt the metastatic cascade have been identified to prevent, shrink or even eradicate metastasis.

As we discussed in this review article, tumor metastasis includes complex mechanisms in which three major factors are interrelated, namely primary soil, seeds, and secondary soil. Besides, multiple complex molecular pathways may be involved in one step of metastatic cascades, so a suppressed pathway may be replaced by many alternative routes for the metastasis establishment. Therefore, it should be taken into consideration that therapeutic agents that inhibit a single factor may activate other metastatic factors, leading to unwanted adverse effects. For example, EMT inhibition is demonstrated to stop the primary tumor invasion, whereas the distant disseminated cancer cells may benefit from this approach, accelerating MET and thus facilitating metastatic colonization. Also, blocking angiogenesis can exacerbate the hypoxia microenvironment, activating downstream metastasis-associated pathways. In addition, only targeting one single phase of the metastatic cascade may be insufficient, since there are lots of alternative routes for myriad tumor cells to disseminate. Future therapeutic approaches can use multi-phase targeted metastasis inhibition for a reference and take the whole process of metastatic cascade into consideration. In more detail, an ideal anti-metastasis nanosystem should act on the primary soil to prevent seeds detaching and disseminating, meanwhile, it is capable of blocking the interactions between seeds and secondary soil. As a result, both the early and late stages of the metastatic cascade can be cut off and the chance of tumor metastasis is significantly reduced. As for the established metastatic tumors, cocktail therapy may still be the best option, which holds a better chance to eradicate the metastasis, with complementary efficacy and low risks of recurrence. In other words, for patients with tumors that have not yet metastasized, mitigating tumor cells invasion and migration can reduce metastatic potential, thereby preventing metastasis. For patients with limited and treatable metastasis, targeting the metastatic colonization can inhibit the additional exacerbation.

Beyond innovative work and encouraging outcomes demonstrated in preclinical study, there have been a few nanosystems approved clinically for tumor metastasis treatment, such as liposomal irinotecan (post-gemcitabine metastatic pancreatic cancer), liposomal doxorubicin (metastatic breast cancer), cytarabine liposomes (leptomeningeal metastasis), vincristine sulfate liposomes (metastatic melanoma), etc[8]. Excitingly, more and more anti-metastasis nano-therapeutics is approved for clinical trials, including active-targeted DDS, nanomedicine-enabled gene therapy and immunotherapy[8]. For instance, Atu027, a liposomal siRNA delivery system targeting protein kinase N3 (PKN3), has entered phase II in combination with standard gemcitabine treatment for patients with advanced or metastatic pancreatic adenocarcinoma[168,169]. Besides, phase I/II study is in progress to assess a nano-vaccine that co-delivers recombinant HER2 (dHER2) antigen and AS15 adjuvant to patients with metastatic breast cancer[170]. Obviously, the clinical translation of nanomedicine for anti-metastasis has received considerable critical attention and nanomedicine holds great potential in improving the prognosis of patients with tumor metastasis.

To conclude, with growing efforts dedicated in unveiling the underlying mechanisms of the metastatic cascade, more targetable targets as well as stages will be explored to enrich our therapeutic strategies. Besides, constantly emerging multifunctional novel nanoscale DDS will provide more powerful weapons for us to fight against tumor metastasis.

Acknowledgments

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Author contributions

Qin He designed the review. Junyu Wu searched references and wrote the manuscript with assistance of Man Li. Qin He, Man Li and Yang Long revised the manuscript. All authors have read and approved the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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