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

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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. 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. 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.

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. 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. 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. 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). 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. 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, or by the surface modified ligands that specifically recognize the biomarkers on tumor cells. Due to the unique chemical and physical properties, a single nanoparticle may simultaneously perform both diagnostic and therapeutic functions.

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. Besides, some drugs that exhibited significant activity in preclinical models were restricted by their poor pharmacokinetic properties, resulting in failure in clinical trials. 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. 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. 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. Overall, tumor metastasis can be concluded into complex communication among these three individual factors. 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 micro-environment of foreign tissue; 7) restore the ability of malignant proliferation and form metastasis (metastatic colonization).

![Figure 1](image-url) 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 soil22 (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 factors23. 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 126-59).

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 niche60. Indeed, it has been demonstrated in experimental models that up-regulated HIF would increase the metastatic potential of tumor cells61,62. In addition, hypoxia is also closely related to the resistance of chemotherapy, radiation therapy and photodynamic therapy (PDT), resulting in compromised efficacy and poor prognosis63,64.

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\textsubscript{2}MnO\textsubscript{2}) nano-platform loaded with chlorine e6, a photodynamic agent, and doxorubicin. The MnO\textsubscript{2} nanostructure could not only achieve controlled release of drug in response to H\textsuperscript{+} and glutathione (GSH) within the tumor microenvironment (TME), but also decompose tumor endogenous H\textsubscript{2}O\textsubscript{2} to relieve hypoxia microenvironment. The Mn\textsuperscript{2+} ions generated by reaction of MnO\textsubscript{2} with H\textsuperscript{+} 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 blockade. 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 chemophotodynamic 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.
suggesting that H–MnO₂ nano-platform was a promising tool to prohibit metastasis (Fig. 3). To achieve efficient tumor-targeted oxygen delivery, Zhou et al. 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-knockdown strategy was proposed by Li et al., 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 anaerobic 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. 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. Indeed, it has been also reported that tumor-targeted delivery of sodium bicarbonate could elevate the intratumoral pH and promote cellular uptake of...
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 \(^{34}\).

**Figure 3** The abscopal effect of H–MnO\(_2\)-PEG/C&D in combination with anti-PD-L1 (α-PD-L1) checkpoint blockade. Schematic illustration of H–MnO\(_2\)-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–MnO\(_2\)-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. Copyright © 2017 Springer Nature.
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. Various nanosystems have exhibited excellent anti-angiogenesis and anti-tumor activities in the past few years. Yet, it should also be taken into consideration that anti-angiogenesis may elicit hypoxia, which contributes to tumor aggressiveness and metastasis. Such being the case, vasculature normalization rather than inhibition should be regarded as a paradigm in terms of treating metastasis. To achieve this goal, anti-angiogenesis agents should be carefully controlled in dosages or combined with cytotoxic therapeutic approaches like chemotherapy and PDT.

Additionally, platelets, small bioactive fragments of cytoplasm released from mature megakaryocytes of the bone marrow, also participate in the metastatic cascade. 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. Targeted delivery of platelet inhibitors by nano-DDS is a promising strategy to make up for the deficiency. Zhang et al. 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 effect 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. 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

Extracellular matrix (ECM) is a sophisticated 3D network composed of fibrous proteins, glycoproteins, enzymes, and matricellular proteins. The ECM of primary tumor plays a pivotal role in metastatic initiation and angiogenesis. 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. 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. Marimastat, a broad-spectrum MMPI with poor water solubility, was assembled with hyaluronic acid (HA)-paclitaxel (PTX) prodrug into nanoparticles for metastatic cancer treatment. 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. Besides, significant fibrosis and musculoskeletal syndrome were observed due to the wide-reaching effects, resulting failure in clinical trials. 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. After binding to integrins or LN receptors of tumor cells, it would self-assemble into fibrils. Inspired by the special property of laminin, a biomimetic device based on laminin-mimic peptide was developed to construct artificial ECM, which could significantly inhibit tumor invasion and metastasis (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. As one of the most abundant components of tumor stroma, CAFs are therefore conspicuous targets. Zhao et al. 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. To eliminate the potential risks, a CAFs-targeting nanosystem (PNP/siCXCL12/mAb) was devised to deliver siRNA to silence CXCL12, which maintains the activated phenotype of CAFs and participates in tumor progression and metastasis as we demonstrate above. 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. 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. 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., 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. 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. EMT is demonstrated to be induced by a wide range of agents including WNT, HIF-1α, TGF-β and even cytotoxic drugs. Intervening EMT may serve a purpose to inhibit the initiation of metastasis, so Huang et al. constructed ZnAs@SiO2 nanoparticles, which could promote SHP-1 while inactivate JAK2/STAT3 pathways, thus regulating the underlying gene networks and co-ordinately 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. delivered DOX and a TGF-β receptor inhibitor via hydroxylethyl starch-poly lactate (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.

Besides EMT, mesenchymal to epithelial transition (MET), which is the reversion of EMT, also participates in metastatic cascades. For the disseminated cancer cells, MET may facilitate metastatic colonization. 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. 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. 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. 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. To block the “backdoor", a specific COX-2 inhibitor was co-delivered with DOX to enhance antitumor activity while eliminating CSCs. 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. Even though they can adhere to platelets or form CTC clusters to protect themselves from damages, only a small number of CTCs will survive to extravasate and seed in distant organs. 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. Moreover, CTCs are inversely associated with the seeds that detach from primary soil with potential to seed in distant organs. 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. devised platelet membranes coated nanoparticles, named nanoplatelets, loaded with indocyanine green as well as DOX to track and eliminate CTCs in vasculature (Fig. 5).

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

| Nanocarriers                  | Modification                                      | Payload                          | Strategy                                      | Ref. |
|-------------------------------|---------------------------------------------------|----------------------------------|-----------------------------------------------|------|
| Magnetic core 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-octadecanolic 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 tetrahedron            | 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  |
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. With this point in mind, Kang et al. 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. Another platelets-CTCs interaction blocking strategy was proposed by our group, 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. 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.

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, 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. 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. 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. Current reports on modulating PMN have shown encouraging therapeutic outcomes, indicating that PMN can be regarded as a promising target of tumor metastasis.
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. Ye et al. 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. 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). To release the inflammatory microenvironment and further modulate PMN, Jiang et al. 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 overexpressed 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 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-a-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 downstream 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. Another biomimetic strategy utilized the organ-tropic nature of exosomes to target PMN. By coating with breast cancer cells-
derived exosome membranes, Zhao et al.\textsuperscript{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 nano-vehicles, 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\textsuperscript{11,13,14,16,17,18}).

For example, our group\textsuperscript{11,14,17,18} developed a novel multi-functional anti-metastasis micellar nanovehicle, which can be

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**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 \( \mu \)g), 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 \( \mu \)m. (f) Flow cytometry analysis and 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 \pm 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.
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. Independent of its anti-coagulant activity, LMWH has many other pharmacological properties, including but not limited to anti-tumor and anti-metastasis. The anti-metastasis activity of LMWH can be attributed to its ability to bind to growth factors and adhesion

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**Figure 7** (A) Schematic illustration of PLT/DOX/oGC 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.

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**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 | Inhibit the expression of MMP-9 in tumor cells | Metastatic initiation and dissemination | 124 |
| PBA-LMWH-TOS micellar nanoparticle loaded with doxorubicin | Cut off tumor cell—platelets interactions | Metastatic initiation and dissemination | 41 |
| Peptide-based self-assembling TF siRNA delivery system | Knock down TF expression in both TME and CTCs | Metastatic initiation and dissemination | 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 |
| EpCAM and CD44 dual targeting mesoporous silica nanoparticle loaded with doxorubicin | Eliminate CTCs | Metastatic dissemination and colonization | 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 |
| PBA-LMWH-TOS nanoparticle loaded with an immunopotentiator and doxorubicin | Reverse the immunosuppressive TME | Metastatic dissemination and colonization | 146 |
| | Inhibit CTCs implantation | Metastatic dissemination and colonization | 147 |
| | Interfere the PMN-tropic recruitment and vascular destruction of G-MDSCs | Metastatic dissemination and colonization | 147 |
| | Down-regulate MMP-9 expression of G-MDSCs | | 147 |
proteins, among which selectin is a key mediator of tumor cell-platelet and tumor cell-endothelial cell interactions. 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 neutrophil membrane-associated protein cocktails were preserved to maintain the binding ability. The surface-anchored protein cocktails including LFA-1, L-selectin and β1 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. 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

| Day 3 | 6 | 9 | 12 | 13 | 14 | Day 24 Sacrifice |
|-------|---|---|----|----|----|-----------------|
| B16F10 back inoculation | B16F10 vein injection |

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

7. Targeting established metastasis with nanodDS

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. As a result, different levels of biomarkers are observed between primary and metastatic tumors. 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. 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. 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. Wen et al. 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. Liu et al. 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-

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 (lactic-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.
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. 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. 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 sub-therapeutic 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. 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. Excitingly, more and more anti-metastasis nano-therapeutics is approved for clinical trials, including active-targeted DDS, nanomedicine-enabled gene therapy and immunotherapy. 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. 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. 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.

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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.

References

1. Fidler IJ, Kripke ML. The challenge of targeting metastasis. Cancer Metastasis Rev 2015;34:635–41.
Nanomedicine for metastatic cascade inhibition by interfering with “seed and soil”

2. Tohme S, Simmons RL, Tsang A. Surgery for Cancer: a trigger for metastases. Cancer Res 2017;77:1548–52.

3. Karagiannis GS, Condeelis JS, Oktay MH. Chemotherapy-induced metastasis: mechanisms and translational opportunities. Clin Exp Metastasis 2018;35:269–84.

4. Weber GF. Why does cancer therapy lack effective anti-metastasis drugs?. Cancer Lett 2013;328:207–11.

5. Li L, Tang P, Li S, Qin X, Yang H, Wu CH, et al. Notch signaling pathway networks in cancer metastasis: a new target for cancer therapy. Med Oncol 2017;34:180.

6. Luo C, Lim JH, Lee Y, Granter SR, Thomas A, Vazquez F, et al. A PGClα-mediated transcriptional axis suppresses melanoma metastasis. Nature 2016;537:422–6.

7. Li C, Wang JC, Wang YG, Gao HL, Wei G, Huang YZ, et al. Recent progress in drug delivery. Acta Pharm Sin B 2019;9:1145–62.

8. Shi JJ, Kantoﬀ PW, Wooster R, Farokhzad OC. Cancer nanomedicine: progress, challenges and opportunities. Nat Rev Cancer 2017;17:20–37.

9. Acharya S, Sahoo SK. PLGA nanoparticles containing various anticancer agents and tumour delivery by EPR effect. Adv Drug Deliv Rev 2011;63:70–83.

10. Golombok SK, May JN, Theek B, Appold L, Drude N, Kiessling F, et al. Tumor targeting via EPR: strategies to enhance patient responses. Adv Drug Deliv Rev 2018;130:17–38.

11. Lavrador P, Gaspar VM, Mano JF. Stimuli-responsive nanocarriers for delivery of bone therapeutics—barriers and progresses. J Control Release 2018;273:51–67.

12. Spicer CD, Jumeaux C, Gupta B, Stevens MM. Peptide and protein nanoparticle conjugates: versatile platforms for biomedical applications. Chem Soc Rev 2018;47:5574–620.

13. Kim H, Park Y, Stevens MM, Kwon W, Hahn SK. Multifunctional hyaluronate—nanoparticle hybrid systems for diagnostic, therapeutic and theranostic applications. J Control Release 2019;303:55–66.

14. Steeg PS. Targeting metastasis. Nat Rev Cancer 2016;16:201–18.

15. Hardan I, Weiss L, Hershkovitz R, Greenspoon N, Alon R, Cahalon L, et al. Inhibition of metastatic cell colonization in murine lungs and tumor-induced morbidity by non-peptidic Arg-Gly-Asp mimetics. Int J Cancer 1993;55:1023–8.

16. Kim KB, Prieto V, Joseph RW, Diwan AH, Gallick GE, Papadopoulos NE, et al. A randomized phase II study of cilengitide (EMD 121974) in patients with metastatic melanoma. Melanoma Res 2012;22:294–301.

17. Sanchez-Laorden B, Viros A, Girotti MR, Pedersen M, Saturno G, et al. Tumor-microenvironment in lung cancer-metastasis and its relationship to potential therapeutic targets. Cancer Treat Rev 2014;40:558–66.

18. Ren B, Cui M, Yang G, Wang HY, Feng MY, You L, et al. Tumor microenvironment participates in metastasis of pancreatic cancer. Mol Cancer 2018;17:108.

19. Lambert AW, Pattabiraman DR, Weinberg RA. Emerging biological principles of metastasis. Cell 2017;168:670–91.

20. Singh S, Varney M, Singh RK. Host CXCR2-dependent regulation of melanoma growth, angiogenesis, and experimental lung metastasis. Cancer Res 2009;69:411–5.

21. Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev 2006;25:9–34.

22. Hao Y, Baker D, Ten Dijke P. TGF-β-mediated epithelial-mesenchymal transition and cancer metastasis. Int J Mol Sci 2019;20.
43. Lv YQ, Zhao XM, Zhu LD, Li SJ, Xiao QQ, He W, et al. Targeting intracellular MMPs efficiently inhibits tumor metastasis and angiogenesis. Theranostics 2018;8:2830–45.

44. Hu XX, He PP, Qi GB, Gao YJ, Lin YX, Yang C, et al. Transformable nanomaterials as an artificial extracellular matrix for inhibiting tumor invasion and metastasis. ACS Nano 2017;11:4086–96.

45. Zheng F, Parayath NN, Ene CI, Stephan SB, Koehne AL, Coon ME, Arvizo RR, Saha S, Wang E, Robertson JD, Bhattacharya R, Hu XX, He PP, Qi GB, Gao YJ, Lin YX, Yang C, et al. Therapeutic nanoparticle. ACS Nano 2019;13:12357–71.

46. Li M, Li MM, Yang YL, Liu YK, Xie HB, Yu QW, et al. Remodeling materials and enhance pancreatic cancer therapy. Bio-materials 2018;159:215–28.

47. Lang JY, Zhao X, Qi YQ, Zhang YL, Han XX, Ding YP, et al. Reshaping prostate tumor microenvironment to suppress metastasis via cancer-associated fibroblast inactivation with peptide-assembly-based nanosystem. ACS Nano 2019;13:6700–5.

48. Kovács D, Igan N, Marton A, Rónávári A, Belétey P, Bodai L, et al. Core-shell nanoparticle suppresses metastasis and modify the tumour-supportive activity of cancer-associated fibroblasts. J Nanobiotechnol 2020;18:18.

49. Arvizo RR, Saha S, Wang E, Robertson JD, Bhattacharya R, Mukherjee P. Gold nanoparticle transforms activated cancer-associated fibroblasts to quiescence. ACS Appl Mater Interfaces 2019;11:26060–8.

50. Kovács D, Igan N, Marton A, Rónávári A, Belétey P, Bodai L, et al. Core-shell nanoparticle suppresses metastasis and modify the tumour-supportive activity of cancer-associated fibroblasts. J Nanobiotechnol 2020;18:18.

51. Arvizo RR, Saha S, Wang E, Robertson JD, Bhattacharya R, Mukherjee P. Inhibition of tumor growth and metastasis by a self-therapeutic nanoparticle. Proc Natl Acad Sci U S A 2013;110:6700–5.

52. Zhang XL, Zhang XX, Hu HY, Qiao MX, Zhao XL, Deng YH, et al. Targeted delivery of zoledone to tumor-associated macrophages for cancer immunotherapy. Mol Pharm 2019;16:2249–58.

53. Zhang F, Parayath NN, Ene CI, Stephan SB, Koehne AL, Coon ME, et al. Genetic programming of macromolecules to perform anti-tumor functions using targeted mRNA nanocarriers. Nat Commun 2019;10:3974.

54. Li M, Li MM, Yang YL, Liu YK, Xie HB, Yu QW, et al. Remodeling tumor immune microenvironment via targeted blockade of PI3K-γ and CSF-1/CSF-1R pathways in tumor associated macrophages for pancreatic cancer therapy. J Control Release 2020;321:23–35.

55. Huang YQ, Zhou B, Luo H, Mao JJ, Huang Y, Zhang K, et al. ZnAs@SiO2 nanoparticles as a potential anti-tumor drug for targeting stemness and epithelial-mesenchymal transition in hepatocellular carcinoma via SHP-1/JAK2/STAT3 signaling. Theranostics 2019;9:4391–408.

56. Zhou Q, Li YH, Zhu YH, Yu C, Jia HB, Bao BH, et al. Co-delivery of nanoparticles to overcome metastasis promoted by insufficient chemotheraphy. J Control Release 2018;275:67–77.

57. Wang B, Ding YP, Zhao XZ, Han XX, Yang N, Zhang YL, et al. Delivery of small interfering RNA against Nogo-B receptor via tumor-acidity responsive nanoparticles for tumor vessel normalization and metastasis suppression. Biomaterials 2018;175:110–22.

58. Fan JX, Zheng DW, Rong L, Zhu JY, Hong S, Li C, et al. Targeting epithelial-mesenchymal transition: metal organic network nanocomplexes for preventing tumor metastasis. Biomaterials 2017;139:116–26.

59. Li YY, Shi SJ, Ming Y, Wang LL, Li CW, Luo MH, et al. Specific cancer stem cell-therapy by albumin nanoparticles functionalized with CD44-mediated targeting. J Nanobiotechnol 2018;16:99.

60. Li YF, Xiao YJ, Lin HP, Reichel D, Bae Y, Lee EY, et al. In vivo β-catenin attenuation by the integrin α5-targeting nano-delivery strategy suppresses triple negative breast cancer stemness and metastasis. Biomaterials 2019;188:160–72.

61. Liu J, Chang BC, Li QL, Xu LM, Liu XX, Wang GB, et al. Redox-responsive dual drug delivery nanosystem suppresses cancer repulsion by abrogating doxorubicin-promoted cancer stemness, metastasis, and drug resistance. Adv Sci (Weinh) 2019;6:1801987.
100. Fang S, Yu L, Mei HJ, Yang J, Gao T, Cheng AY, et al. Cisplatin nanomedicine for metastatic cascade inhibition by interfering with "seed and soil" hypothesis. *Nat Rev Drug Discov* 2014;13:904–27.

83. Lu PF, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. *J Cell Biol* 2012;196:395–406.

81. Dufour A, Overall CM. Missing the target: matrix metalloproteinase inhibitors in antimetastasis and cancer. *Trends Pharmacol Sci* 2013;34:233–42.

82. Vandebroucke RE, Libert C. Is there new hope for therapeutic matrix metalloproteinase inhibition? *Nat Rev Drug Discov* 2014;13:904–27.

84. Hörker VH, Shewan D, Tessier-Lavigne M, Poo M, Holt C. Growth-cone attraction to netrin-1 is converted to repulsion by laminin-1. *Nature* 1999;401:69–73.

85. Seoane J, Gomis RR. TGF-β family signaling in tumor suppression and cancer progression. *Cold Spring Harb Perspect Biol* 2017;9.

86. De Silva DM, Roy A, Kato T, Cecchi F, Lee YH, Matsumoto K, et al. Targeting the hepatocyte growth factor/Met pathway in cancer. *Biochem Soc Trans* 2017;45:555–70.

87. Knachel S, Anderle P, Werfell P, Diamantis E, Rieggl C. Fibroblast surface-associated FGF-2 promotes contact-dependent colorectal cancer cell migration and invasion through FGFR-SRC signaling and integrin avß6-mediated adhesion. *OncoTarget* 2015;6:14300–17.

88. Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, Williams ED. Mesenchymal-to-epithelial transition facilitates tumor cell invasive properties in microfluidic platforms applied in cancer metastasis: circulating tumor cells (CTCs) isolation and tumor-on-a-chip. *Small* 2020;16:e1903899.

89. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature* 2007;450:1235–9.

90. Myung JH, Gajjar KA, Saric J, Eddington DT, Hong S. Dendrimer-mediated multivalent binding for the enhanced capture of tumor cells. *Angew Chem Int Ed Engl* 2011;50:11769–72.

91. Werner S, Stenzl A, Pantel K. Tumour microenvironmetn. Expression of epithelial-mesenchymal transition and cancer stem cell markers in circulating tumor cells. *Adv Exp Med Biol* 2017;994:205–28.

92. Lin ZJ, Luo GY, Du WX, Kong TT, Liu CK, Liu Z. Recent advances in microfluidic platforms applied in cancer metastasis: circulating tumor cells (CTCs) isolation and tumor-on-a-chip. *Small* 2020;16:e1903899.

93. Oren B, Urosevic J, Mertens C, Mora J, Guiu M, Gomis RR, et al. Paclitaxel therapy promotes breast cancer metastasis in a TLR4-dependent manner. *Cancer Lett* 2016;380:270–82.

94. Liu TR, Xu HN, Huang MG, Ma WJ, Saxena D, Lustig RA, et al. Circulating glioma cells exhibit stem cell-like properties. *Cancer Res* 2018;78:6632–42.

95. Markowska A, Sajad S, Huczynski A, Rehls S, Markowska J. Ovarian cancer stem cells: a target for oncological therapy. *Adv Clin Exp Med* 2018;27:1017–20.

96. Kurtova AV, Xiao J, Mo Q, Pazhanisamy S, Krasnow R, Werner S, et al. Blocking FGE2-induced tumour repopulation abrogates bladder cancer chemoresistance. *Nature* 2015;517:209–13.

97. Wirtz D, Konstantopoulos K, Seaton PC. The physics of cancer: the role of physical interactions and mechanical forces in metastasis. *Nat Rev Cancer* 2011;11:512–22.

98. McCarty OJ, Mousa SA, Bray PF, Konstantopoulos K. Immobilized platelets support human colon carcinoma cell tethering, rolling, and firm adhesion under dynamic flow conditions. *Blood* 2000;96:1789–97.

99. KATO M, Ohishi K, Takeya M. Tumor-associated macrophages promote mesenchymal-like characteristics in osteosarcoma through contact-dependent colorectal cancer cell migration and invasion through FGFR-SRC signaling and integrin avß6-mediated adhesion. *Circulating glioma cells exhibit stem cell-like properties. J Clin Oncol* 2012;30:525–32.

100. Dasgupta A, Lim AR, Ghajar CM. Circulating and disseminated tumor cells: harbinger or initiators of metastasis?. *Adv Drug Deliv Rev* 2017;11:414–22.

101. Liu Q, Zhang HF, Jiang XL, Qian CY, Liu ZQ, Luo DY. Factors involved in cancer metastasis: a better understanding to "seed and soil" hypothesis. *Mol Cancer Therapeut* 2017;16:176.

102. Park HA, Brown SR, Kim Y. Cellular mechanisms of circulating tumor cells during breast cancer metastasis. *Int J Mol Sci* 2020;21.

103. Oren B, Urosevic J, Mertens C, Mora J, Guim J, Gomis RR, et al. Tumour stroma-derived lipocalin-2 promotes breast cancer metastasis. *J Pathol* 2016;238:274–85.

104. Tan Y, Gao RG, Yang J, Wang J, Li X, Tang X, et al. Tumour-associated macrophages promote lung metastasis and induce epithelial-mesenchymal transition in osteosarcoma by activating the COX-2/STAT3 axis. *Cancer Lett* 2019;440–411:16–25.

105. Christofori G. New signals from the invasive front. *Nature* 2006;441:444–50.

106. Demirkan B. The roles of epithelial-to-mesenchymal transition (EMT) and epithelial-to-epithelial transition (MET) in breast cancer bone metastasis: potential targets for prevention and treatment. *J Clin Med* 2013;2:664–82.

107. Clevers H, Nusse R. Wntß-catenin signaling and disease. *Cell* 2012;149:1192–205.

108. Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. *Cell Res* 2009;19:756–72.

109. Van-Dreaper L, Hall K, Griggs C, Rajipur S, Kohlb P, DeNardo D, et al. Paclitaxel therapy promotes breast cancer metastasis in a TLR4-dependent manner. *Cancer Res* 2014;74:5421–34.

110. Fang S, Yu L, Mei HJ, Yang J, Gao T, Cheng AY, et al. Cisplatin promotes mesenchymal-like characteristics in osteosarcoma through Snail. *Oncol Lett* 2016;12:5007–14.

111. Chaffer CL, Brennan JP, Slavin JL, Blick T, Thompson EW, Williams ED. Mesenchymal-to-epithelial transition facilitates bladder cancer metastasis: role of fibroblast growth factor receptor-2. *Cancer Res* 2006;66:11271–8.
123. Gao Y, Xie XD, Li PQ, Lu YS, Li T, Lian S, et al. A novel nano-missile targeting two biomarkers and accurately bombarding CTCs with doxorubicin. Nanoscale 2017;9:5624–40.

124. Yao JH, Feng JX, Gao XL, Wei D, Kang T, Zhu QQ, et al. Neovascularization and circulating tumor cells dual-targeting nanoparticles for the treatment of the highly-invasive breast cancer. Biomaterials 2017;113:1–17.

125. Chen ND, Qin SY, Yang XH, Wang Q, Huang J, Wang KM. Sense-and-treat" DNA nanodevice for syndromic destruction of circulating tumor cells. ACS Appl Mater Interfaces 2016;8:26552–8.

126. Li LX, Schmitt M, Matzke-Ogi A, Wadhwan P, Orian-Rousseau V, Levkin PA. CD44v6-peptide functionalized nanoparticles selectively bind to metastatic cancer cells. Adv Sci (Weinh) 2017;4:1600202.

127. Luk BT, Zhang L. Cell membrane-camouflaged nanoparticles for drug delivery. J Control Release 2015;220:600–7.

128. Hu CM, Zhang L, Aryal S, Cheung C, Fang RH, Zhang L. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. Proc Natl Acad Sci USA 2011;108:10980–5.

129. Luk BT, Hu CM, Fang RH, Dehaini D, Carpenter C, Gao W, et al. Interfacial interactions between natural RBC membranes and synthetic polymeric nanoparticles. Nanoscale 2014;6:2730–7.

130. Park J, Wysocki RW, Amoozgar Z, Maiorino L, Fein MR, Jorns J, et al. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. Sci Transl Med 2016;8:361ra138.

131. Kang T, Zhu QQ, Wei D, Feng JX, Yao JH, Jiang TZ, et al. Nanoparticles coated with neutrophil membranes can effectively treat cancer metastasis. ACS Nano 2017;11:1397–411.

132. Fong MY, Zhou W, Liu L, Alontaga AY, Chandra M, Ashby J, et al. Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. Nat Cell Biol 2015;17:183–94.

133. Zeng ZC, Li YL, Pan YJ, Lan XL, Song FY, Sun JB, et al. Cancer-derived exosomal miR-25–3p promotes pre-metastatic niche formation by inducing vascular permeability and angiogenesis. Nat Commun 2018;9:5395.

134. Huang R, Rofstad EK. Integrins as therapeutic targets in the organ-specific metastasis of human malignant melanoma. J Exp Clin Cancer Res 2018;37:92.

135. Qin Q, Ren L, Jian M, Xu PP, Li J, Zheng P, et al. The mechanism of the premetastatic niche facilitating colorectal cancer liver metastasis generated from myeloid-derived suppressor cells induced by the SIP1R1-STAT3 signaling pathway. Cell Death Dis 2019;10:693.

136. Sakaguchi M. S100-SPECT uncovers cellular and molecular events of pre-metastatic niche formation and following organ-specific cancer metastasis. Theranostics 2017;7:2649–51.

137. Zhang JC, Han XQ, Shi HF, Gao YY, Qiao X, Li JH, et al. Lung resided monocytic myeloid-derived suppressor cells contribute to premetastatic niche formation by enhancing MMP-9 expression. Mol Cell Probes 2020;50:101498.

138. Reiterer M, Colaco R, Emrouznejad P, Jensen A, Rundquist H, Johnson RS, et al. Acute and chronic hypoxia differentially predisposes lungs for metastases. Sci Rep 2019;9:10246.

139. Hiratsuka S, Ishibashi S, Tomita T, Watanabe A, Akashi-Takamura S, Murakami M, et al. Primary tumours modulate innate immune signalling to create pre-metastatic vascular hyperpermeability foci. Nat Commun 2013;4:1853.

140. Huang YJ, Song N, Ding YP, Yuan SP, Li XH, Cai HC, et al. Pulmonary vascular destabilization in the premetastatic phase facilitates lung metastasis. Cancer Res 2009;69:7529–37.

141. Psaila B, Lyden D. The metastatic niche: adapting the foreign soil. Nat Rev Cancer 2009;9:285–93.

142. Goldman E, Zinger A, da Silva D, Yaari Z, Kajal A, Vardi-Oknin D, et al. Nanoparticles target early-stage breast cancer metastasis in vivo. Nanoletters 2017;28:43101.

143. Jiang TZ, Chen L, Huang YK, Wang JH, Xu MJ, Zhou SL, et al. Metformin and docosahexaenoic acid hybrid micelles for premetastatic niche modulation and tumor metastasis suppression. Nano Lett 2019;19:3548–62.

144. Xiong H, Du S, Zhang P, Jiang ZJ, Zhou JP, Yao J. Primary tumor and pre-metastatic niches co-targeting “peptides-legos” hybrid hydroxyapatite nanoparticles for metastatic breast cancer treatment. Biomater Sci 2018;6:2591–604.

145. Xie XD, Nie HF, Zhou Y, Lian S, Mei H, Lu YS, et al. Eliminating blood oncocytic exosomes into the small intestine with aptamer-functionalized nanoparticles. Nat Commun 2019;10:5476.

146. Ye H, Wang KY, Lu Q, Zhao J, Wang ML, Kan QM, et al. Nanosponge of circulating tumor-derived exosomes for breast cancer metastasis inhibition. Biomaterials 2020;242:119932.

147. Long Y, Lu ZZ, Xu SS, Li M, Wang XH, Zhang ZR, et al. Self-delivery micellar nanoparticles prevent premetastatic niche formation by interfering with the early recruitment and vascular destruction of granulocytic myeloid-derived suppressor cells. Nano Lett 2020;20:2219–29.

148. Zhao LW, Gu CY, Gan Y, Shao LL, Chen HW, Zhu HY. Exosome-mediated siRNA delivery to suppress postoperative breast cancer metastasis. J Control Release 2020;318:1–15.

149. Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature 2018;560:382–6.

150. Hiratsuka S, Watanabe A, Sakurai Y, Akashi-Takamura S, Ishibashi S, Miyake K, et al. The S100A8-serum amyloid A3–TLR4 paracrine cascade establishes a pre-metastatic phase. Nat Cell Biol 2008;10:1349–55.

151. Guo R, Long Y, Lu ZZ, Deng M, He PH, Li M, et al. Enhanced stability and efficacy of GEM-TOS prodrug by co-assembly with antimetastasis agent LMWH-TOS. Acta Pharm Sin B 2020;10:977–88.

152. Mullany B, Hogwood J, Gray E, Lever R, Page CP. Pharmacology of heparin and related drugs. Pharmacol Rev 2016;68:76–141.

153. Laubli H, Borsig L. Heparins attenuate cancer metastasis: are selectins the link?. Cancer Invest 2009;27:474–81.

154. Simonis D, Christ K, Alban S, Bendas G. Affinity and kinetics of different heparins binding to P- and L-selectin. Semin Thromb Hemost 2007;33:534–9.

155. Krilleke D, DeErkenez A, Schubert W, Giri I, Robinson GS, Ng YS, et al. Molecular mapping and functional characterization of the VEGF164 heparin-binding domain. J Biol Chem 2007;282:28045–56.

156. Brastianos PK, Carter SL, Santagata S, Cahill DP, Taylor-Weiner A, Jones RT, et al. Genomic characterization of brain metastases reveals branched evolution and potential therapeutic targets. Cancer Discov 2015;5:1164–77.

157. Taipe C, Savic S, Wagner U, Schönegg R, Novotny H, Grilli B, et al. HER2 gene status in primary breast cancers and matched distant metastases. Breast Cancer Res 2007;9:R31.

158. Curtit E, Nerich V, Mansi L, Chaigneau L, Cals L, Villanueva C, et al. Discordances in estrogen receptor status, progesterone receptor status, and HER2 status between primary breast cancer and metastases. Oncol Rep 2013;18:667–74.

159. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366:883–92.

160. Fidler I. The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. Nat Rev Cancer 2003;3:453–8.

161. Zhou YQ, Peng ZL, Seven ES, Leblanc RM. Crossing the blood–brain barrier with nanoparticles. J Control Release 2018;250:290–303.

162. Wen J, Wu D, Qin M, Liu CY, Wang L, Xu D, et al. Sustained delivery and molecular targeting of a therapeutic monoclonal antibody to metastases in the central nervous system of mice. Nat Biomed Eng 2019;3:706–16.

163. Mei L, Rao JD, Liu YY, Li M, Zhang ZR, He Q. Effective treatment of the primary tumor and lymph node metastasis by polymeric micelles with variable particle sizes. J Control Release 2018;292:67–77.

164. Liu J, Li HJ, Luo YL, Xu CF, Du XJ, Du JZ, et al. Enhanced primary tumor penetration facilitates nanoparticle draining into lymph nodes.
after systemic injection for tumor metastasis inhibition. *ACS Nano* 2019;13:8648–58.

165. Yhee JY, Son S, Lee H, Kim K. Nanoparticle-based combination therapy for cancer treatment. *Curr Pharm Des* 2015;21:3158–66.

166. Nam J, Son S, Ochyl LJ, Kuai R, Schwendeman A, Moon JJ. Chemo-photothermal therapy combination elicits anti-tumor immunity against advanced metastatic cancer. *Nat Commun* 2018;9:1074.

167. Song W, Kuang J, Li CX, Zhang M, Zheng D, Zeng X, et al. Enhanced immunotherapy based on photodynamic therapy for both primary and lung metastasis tumor eradication. *ACS Nano* 2018;12:1978–89.

168. Santel A, Aleku M, Röder N, Möpert K, Durieux B, Janke O, et al. Atu027 prevents pulmonary metastasis in experimental and spontaneous mouse metastasis models. *Clin Cancer Res* 2010;16:5469–80.

169. Aleku M, Schulz P, Keil O, Santel A, Schaeper U, Dieckhoff B, et al. Atu027, a liposomal small interfering RNA formulation targeting protein kinase N3, inhibits cancer progression. *Cancer Res* 2008;68:9788–98.

170. Hamilton E, Blackwell K, Hobeika AC, Clay TM, Broadwater G, Ren XR, et al. Phase 1 clinical trial of HER2-specific immunotherapy with concomitant HER2 kinase inhibition [corrected]. *J Transl Med* 2012;10:28.