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Abstract

Primary sites of tumor are the focal triggers of cancers, yet it is the subsequent metastasis events that cause the majority of the morbidity and mortality. Metastatic tumor cells exhibit a phenotype that differs from that of the parent cells, as they represent a resistant, invasive subpopulation of the original tumor, may have acquired additional genetic or epigenetic alterations under exposure to prior chemotherapeutic or radiotherapeutic treatments, and reside in a microenvironment differing from that of its origin. This combination of resistant phenotype and distal location make tracking and treating metastases particularly challenging. In this review, we highlight some of the unique biological traits of metastasis, which in turn, inspire emerging strategies for targeted imaging of metastasized tumors and metastasis-directed delivery of therapeutics.

1. Introduction: the challenge of targeting metastases

The concept of targeted delivery is well established for tumors but has been applied less extensively to metastases. Clearly, the development of effective delivery methods is critical for cancer, for which it is essential to direct as much drug to the cancer cells while sparing healthy cells to the greatest extent possible. Considerable research progress has been made in understanding the challenges and in exploiting the biology of tumors for drug delivery. Passive targeting via the enhanced permeation and retention (EPR) effect and active targeting via integrins, growth factor receptors and other cell surface ligands are well studied approaches for enhancing drug delivery to established tumors [1-3]. While the traditional focus has been on delivering cancer therapeutics to primary tumors, a yet more significant challenge that is only now becoming recognized is targeting secondary tumors. Here, the challenge to eradicating cancers shifts to the identification and elimination of metastases that have spread from the primary tumor site and have the capability to grow new tumors in distant tissues.

Indeed, 90% of cancer deaths come from metastasis [4]. Metastases are frequently multifocal, are notoriously difficult to eradicate, and consequently have low response and cure rates [5]. They result from the summation of a series of low probability events: the acquisition of stem cell-like properties by the primary tumor cells, shedding and dissemination to a distant tissue, colonization mediated by bidirectional signaling between tumor cell and stroma, a period of dormancy, and eventually reawakening of micrometastases and outgrowth of full-blown metastatic disease [6]. The inherent selection process required for this process results in metastases that are separated in time and space from the primary tumor, are often present at multiple sites,

and that are endowed with genetic and epigenetic alterations that render them resistant to treatment and often even tracking [7, 8].

Metastases present unique challenges from a drug delivery standpoint. In order to head off the morbidity and mortality associated with metastatic disease, targeting micrometastases before they manifest themselves in overt disease and/or spread further is necessary. One consequence is that reliance upon the EPR effect for drug accumulation is unlikely to be effective for metastases whose vasculature is not yet developed. Once established, metastases have characteristics of host tissue and primary tumor that may complicate targeting strategies that would be effective for the primary tumor. As such, novel approaches to the sensitive and accurate detection of microlesions are needed. These will require sophisticated targeting strategies that allow an imaging agent to find and discriminate the microlesion from the host environment. At the same time, it may be possible to exploit the dual nature of metastases, with characteristics of both tumor (seed) and stroma (soil), for effective targeting [9].

In this review, we outline emerging strategies and approaches for targeted delivery of agents to metastases for the integrated purposes of detection, tracking and therapy. First, we review the main features of metastasis with a focus on features that may be utilized for targeted detection and delivery. Second, we explore mechanisms by which the metastatic process itself may be targeted. Third, we review approaches that have been developed for targeting metastases that have taken hold in particular organs and are beginning to emerge. Fourth, we motivate the need for and application of targeting approaches to high resolution imaging of metastases and metastatic processes.

2.The biology of metastasis: implications for delivery and tracking

Metastasis, a multistage process, involves spread of malignant cells from primary tumor to distant organs in new microenvironments [10]. Jean Claude Recamier in 1829 was the first to reference and document metastasis - "métastase", hematogenous spread of disease [11]. Stephen Paget's seed and soil theory of metastases [12], stating that a permissive microenvironment (the soil) promotes growth of the disseminated tumor cell (the seed), remains the basis of research to date and is widely accepted to explain the mechanism of metastasis. The "metastatic cascade", a phenomenon first reported in 1975, involves distinct steps involving local invasion, intravasation into adjacent blood and lymphatic vessels, transit through circulation and evasion of host immune systems, extravasation into the parenchyma of distant organs, and colonization and formation of micro-metastases, followed by proliferation and progression to macro-metastases (Figure 1) [13-15]. Understanding the molecular basis of interaction between primary tumor and distant metastases and their niche will be central to designing distinctive molecularly targeted strategies for the primary versus metastatic tumors.
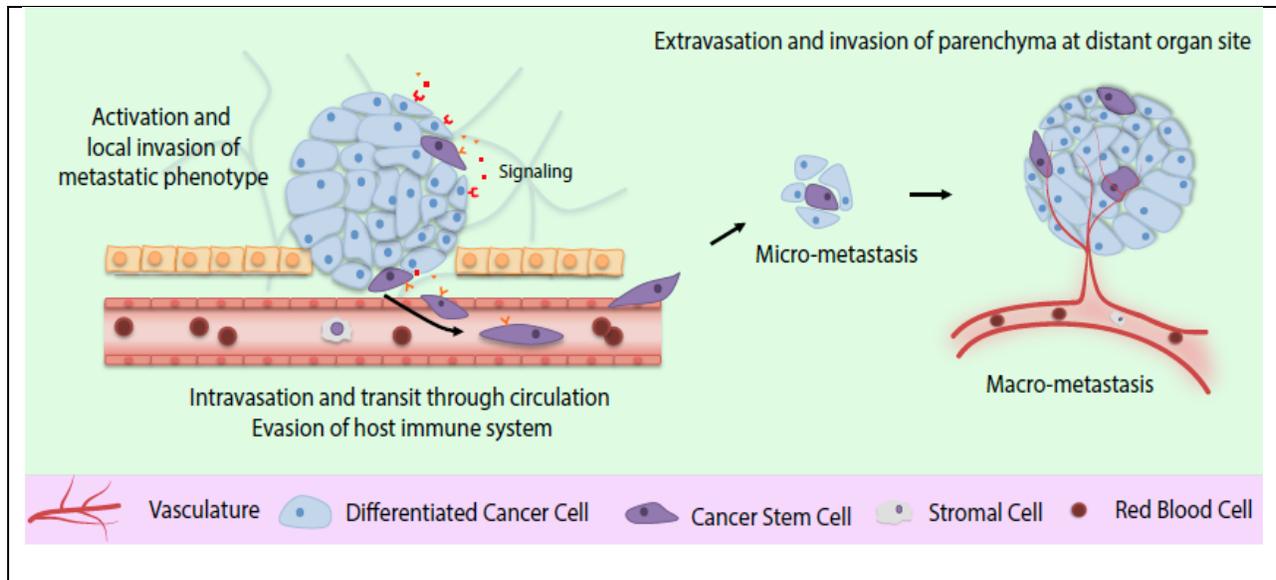
Figure 1: The metastatic cascade -- from primary tumor to macrometastases. Beginning with tumor initiating (stem) cells, a stepwise progression occurs from the tumors exiting the primary site of growth (local invasion and activation of metastatic phenotype) followed by systemic translocation (intravasation and transit through circulation) and finally adaptation and reawakening in distant metastatic sites (extravasation, micrometastases formation and colonization leading to clinically detectable active macrometastases). The metastatic process requires activation of cancer stem cells and interaction with surrounding stromal cells throughout the program.

Current thinking says that heterogeneity within a tumor, both physical and functional in nature, can be explained by the cancer stem cell model [16, 17]. One of the posits of the model is that cancer stem cells, a minor fraction of all tumor cells, drive tumor progression though either innate or acquired therapy resistance and by formation of metastases [18, 19]. Cancer cell heterogeneity is frequently explained by the clonal evolutionary theory [20, 21], though this does not encompass the heterogeneity that arises from genetic evolution and epigenetic changes. Theoretically, cancer stem cells (CSCs) can arise from the cell-of-origin of tumor or from transformed cells within the parent tumor as a result of genetic and epigenetic alterations, as has been studied in a diverse range of cancers, including glioblastoma multiforme [22-24], prostate cancer [25-29], colorectal cancers [30-36] and breast cancers [37-39] models, among others.

However, due to patient-to-patient variability and lack of consistent results from xenograft models, the cancer stem cell model is not universally accepted.

During each step in the metastatic cascade, tumor cells interact with their immediate non-tumor microenvironment. For instance, the interaction of disseminating tumor cells in circulation with macrophages, leukocytes and other immune components has been studied in depth and form the basis for molecularly targeted therapies [40-44]. The molecular signaling between tumor and stromal cells is only beginning to be unraveled. The physical microenvironment, e.g. flow in the case of circulating tumor cells, also plays a major role in the adaptation that disseminating cells must undergo and the phenotypes that they acquire [45].

In metastases, the interaction of specialized cancer cell(s) ("seed") and the host ("soil") promotes the emergence of a metastatic phenotype that is evolved from that of the parent tumor. Numerous studies have shown the molecular differences between primary and distant metastases affecting treatment decisions. For instance, variable Her2 expression levels in primary tumors lead to subsequent clonal expansion of Her2- populations and a discordance in Her2 levels between the primary tumor and distant metastases in breast cancer [46]. Similar discordance in ER and PR receptor expression has been observed between the primary breast tumor and the corresponding metastatic lesions [47]. In colorectal cancer patients with wild-type KRAS, failure of EGFR antibody therapy was observed due to activating BRAF or PIK3CA mutations underlying intratumoral heterogeneity. Increased heterogeneity between primary tumors and lymph node metastases presented a major challenge for

targeted EGFR therapy as a choice for these patients [48]. The basic question of how seed and soil heterogeneity synergize to promote metastases remains unanswered.

Finally, inter-tumor heterogeneity (between tumors of the same tissue type arising in different patients) and intra-tumor heterogeneity (within a single patient tumor) additionally pose challenges in identification of effective cancer biomarkers, prediction of treatment response, and the design of targeted therapies. Concepts of cancer stem cell biology and Paget's theory will need to be adapted to emerging new technologies and experimentally testable hypotheses with clinical relevance developed for targeting tumors.

Given their origin from interactions between "seed" and "soil," metastases may exhibit phenotypic properties partially of the parent tumor, partially of the host tissue and potentially novel properties resulting from the unique interaction of tumor cells with their new host. As a result, a therapy that might have had excellent efficacy on the parent tumor might be ineffective on metastases. On the other hand, understanding the interactions of metastases with their host might provide opportunities for targeting a therapy to the correct cells with fairly high specificity. That is, a metastasis might be targeted based on the properties of tumor cells themselves, based on the mechanics of the metastatic process, or based on characteristics of the host tissues. These strategies are discussed further in the following sections.

3. Targeting metastatic processes

The successes and limitations of molecularly targeted therapies can inform the development of targeted delivery strategies for metastasis. One of the drivers toward precision medicine has been an explosive growth of promising targets identified in the

past decade resulting from extensive understanding of metastatic tumor biology. The predominant targets include growth factor receptors [49-51], tyrosine and serine-threonine kinase receptors [52-54] and non-receptor signaling molecules [41, 55, 56], which drive cancer cell survival, proliferation, and progression. Herceptin, a monoclonal antibody against receptor tyrosine kinase HER2 (ErbB2) was the first targeted therapy, approved in 1998 by the FDA for treating patients with HER2-positive metastatic breast cancer [50, 57]. This was closely followed by the Bcr-Abl targeted drug, Gleevec, for chronic myeloid leukemia [58-61]. Although the FDA has since approved a large number of drugs developed against a range of both therapeutic and non-therapeutic targets, it has become apparent that a single targeted therapy is not sufficient to cure most cancers, due in significant part to inter-tumor heterogeneity and to drug resistance associated with heterogeneity of metastases. Thus, detection of and delivery to nascent and established metastases remain as critical barriers to reaping the benefits of precision medicine and to cancer therapy in general.

A precursor to targeted delivery to disseminated metastases is the use of immunoconjugates for treating leukemias. For instance, Mylotarg was developed as a conjugate of the potent antitumor antibiotic, calicheamicin, with the anti-CD33 antibody. Similarly, Zevalin [62] and Bexxar [63] employed anti-CD20 antibodies to target radioisotopes to cancer cells. These agents, though clinically effective, exhibited lethal side effects most likely due to non-specific binding between the targeting agent and non-target moieties on the cell surface and possible interaction with the target expressed on non-cancerous cells.

The targeting paradigm can be adapted to metastases by addressing unique site tags on the populations of cells that form metastases or by exploiting the metastatic phenotype. The recent explosion of research on cancer stem cells (CSCs), in addition to modifying how we think about resistance and metastasis, has brought to fore several cell surface markers that can be exploited to deliver therapeutic agents to this otherwise hard-to-reach subpopulation of cells. The most commonly applied approach has centered on hyaluronic acid (HA), which is a natural glycosaminoglycan and intrinsic ligand for CD44 expressed on many cancer stem cells. HA has been functionalized to a variety of nanoparticles and used to target and increase the effectiveness of drug delivery in mammary fat pad and lung metastasis models [64, 65]. Another common cancer stem cell marker is CD133, which does not have an easily utilized natural ligand. Nonetheless, it has been targeted for nanoparticle drug delivery in mouse models using an evolved aptamer and a monoclonal antibody as ligands [66, 67]. While not exclusive to cancer stem cells, EGFR is a common oncogene, and the anti-EGFR monoclonal antibody cetuximab has been approved for treatment of metastatic colon cancer and head and neck squamous cell carcinoma. This antibody is being investigated as a means to target nanoparticles administered via convection-enhanced delivery to glioblastoma with the intent of reaching glioma CSCs [68]. Other cell surface markers such as EpCAM may emerge as additional receptors for targeted delivery to CSCs [69].

From a physical standpoint [4, 45], the metastatic process can be conceptualized in terms of a series of steps including detachment, intravasation, circulation, extravasation, colonization and eventually reactivation. The molecular basis for each of these processes is at least partially understood and provides a potential avenue for

delivering therapeutic agents. For example, growth factors, chemokines, integrins and matrix metalloproteinases are all viable targets for cell motility, and indeed motility inhibitors are in clinical development [70]. Integrins, which are frequently associated with angiogenic tumors, are also involved in cellular invasion, as the invasive cell switches from cell-cell to cell-ECM interactions. This interaction has been targeted using RGD peptides conjugated to either liposomes or albumin nanoparticles to modulate metastasis in vivo [71, 72]. Cell surface receptors associated with motility, such as CXCR4, represent another targeting option for delivery of drugs and siRNA [73]. Likewise, nanoparticles carrying a substrate for MMPs have been used to target invading cell populations [74].

Once a tumor cell has reached the circulation, it is a hard-to-find entity in the 3 L of plasma circulating in the average human. Nonetheless, targeting circulating tumor cells (CTCs) in circulation provides an opportunity to prevent seeding of distant metastases [75]. A number of advances have been made toward isolating and characterizing circulating tumor cells, by combining the technologies of microfluidic devices and biomolecular recognition via adhesion ligands and/or tumor cells markers, for diagnostic purposes. It has been suggested that similar technology employing magnetic nanoparticles could be used to capture CTCs and reduce their in vivo burden [76]. An alternative approach is to "functionalize" leukocytes by attachment of nanoparticles (via selectins) for homing to CTCs and presentation of death ligands [77].

The ultimate landing of tumor cells in a distant tissue is mediated by adhesion ligands that can capture cells in flow, most notably the selectins. Nanoparticles functionalized with selectins could thus be used to capture circulating tumor cells [78] or

metastases that have sprouted in distant tissues [79]. In order for such a strategy to be effective, nanoparticles would need to be long circulating. This has been achieved in various applications by extensive functionalization with PEG or by creating flexible, disc-like nanoparticles that mimic red blood cells and share their flow and adhesion characteristics in the circulation [78, 80].

4. Targeting organ-specific metastases

The concept that metastases exhibit organ specificity was first described in 1980 [81]. Owing to differences in barriers of infiltration and the microenvironment of individual distant metastatic sites, metastatic propensities and dynamics to different organs can vary. Bone marrow, lungs, liver and brain are the most common clinically relevant sites of metastases. From an infiltration perspective, fenestrated sinusoid capillaries of bone marrow are more permissive to cancer cell infiltration than the contiguous structure of lung capillary walls and brain capillaries, which are more difficult to penetrate. Infiltration through these barriers selects for those tumor cells that express the necessary extravasation functions. Thus, in colorectal carcinoma, the mesenteric circulation from the bowels and the permissiveness of the liver capillary sinusoids are thought to favor liver metastasis [82, 83]. However, to cross the blood-brain barrier (BBB) and metastasize to the brain, cancer cells require specialized mechanisms [84-88]. Thus, therapeutic agents could conceivably be designed to target these organ systems via mimicry of the mechanisms utilized by cancer cells during the metastatic process (Figure 2).
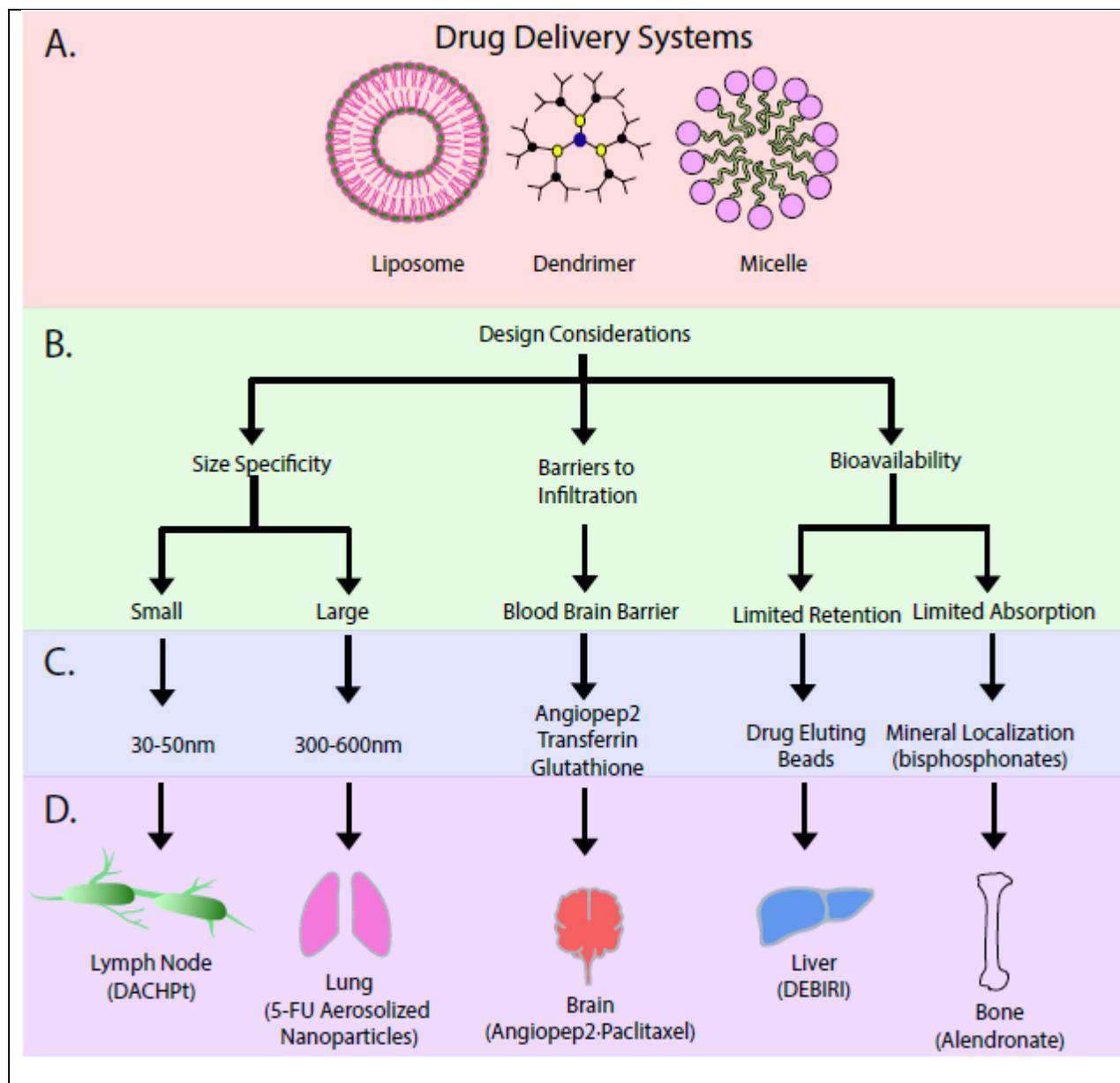
Figure 2: Drug delivery systems for targeting organ-specific metastases. **A.** Nanoarchitectures provide sizes favorable for delivery as well as the presentation of multiple targeting ligands for increased avidity and the concentration of payload. **B.** In targeting a particular tissue, design considerations include the filtering characteristics of the organ and specialized physiological barriers that hinder uptake. **C.** These considerations lead to a restricted set of variables to be optimized experimentally, and **D.** implemented for successful targeting and imaging/eradication of metastases.

Metastasis to Lymph Nodes: A variety of cancers initiate their metastatic spread via the lymphatic system. Nanoparticles can be used to extend the circulation time of therapeutics such that they distribute to and reach the lymphatics [89]. A number of

studies have suggested that the lower size end of therapeutic nanoparticles may be beneficial for this application. For example, sub-50 nm polymeric micelles have been used to deliver platinum-based chemotherapies to lymph and brain metastases [90, 91], while ~15 nm particles were used to deliver doxorubicin successfully to breast cancer metastases in the lymph [92]. In addition to size, targeting of lymph can be accomplished using the LyP-1 peptide, which localizes with lymphatic endothelium and tumor associated macrophages. For example, LyP-1 has been conjugated to PEGylated liposomes for delivery of doxorubicin leading to inhibition of lymph metastases [93]. More generic tumor targeting motifs such as the integrin-targeting RGD peptide have also demonstrated effectiveness is delivery of therapeutics to minimize metastatic formation and growth in lymph [94]. A promising new avenue is the targeting of lymphatics with nanoparticles presenting antigens and adjuvants for immunotherapy [95].

Metastasis to the Lungs: Drug delivery for treatment of lung metastases can be achieved by taking advantage of the large surface area, thin alveolar epithelium, rapid absorption, lack of first-pass metabolism, high bioavailability, and the capacity to absorb large quantities of drug of the lung. The first study with 5-fluorouracil delivered in a nebulized form showed higher accumulation of the drug in the tumor than in the surrounding normal tissue [96]. Several studies, clinical and preclinical, are underway to explore aerosol chemotherapy [97] using nanoparticles as a means of targeting lung metastases [98-103]. Aerosol administration can be coupled with molecularly targeted therapy, as demonstrated using gelatin based nanoparticle formulation targeting epidermal growth factor receptors in mice [104-106]. Systemic delivery of drugs to lungs

can be improved using vehicles that extend circulation time, such as small cationic DOTAP liposomes with nitric oxide synthase gene and low-dose cisplatin [107]. Furthermore, molecularly targeted liposomal and polymeric nanoparticle formulations have been applied to lung metastases [108, 109].

Metastasis to the Brain: Brain metastases are most commonly seen from cancers of the lung, breast and skin (melanoma) [110]. The development of brain-targeted therapeutics and chemotherapeutics has been extremely challenging due to limited permeability of the blood-brain barrier (BBB). Nonetheless, some agents that mediate passage across the BBB have been identified and exploited. For example, angiopep-2, a 19-amino acid peptide that binds the low-density lipoprotein receptor related protein (LRP) receptors at the BBB, was conjugated to paclitaxel and greater than 50-fold enhanced delivery across the BBB was observed [111]. This agent has now entered clinical trials. Conjugates of angiopep-2 with molecularly targeted therapies such as anti-HER2 monoclonal antibodies are emerging [112]. Alternative ligands that mediate passage across the BBB include transferrin and glutathione. Of note, glutathione-conjugated PEGylated liposomes have been utilized in preclinical and clinical studies for delivery of numerous agents to treat a variety of central nervous system disorders, including brain metastases. Recent Phase 1/2a clinical trial results from the use of this platform for doxorubicin delivery against brain metastases are promising [113].

A number of other approaches are under investigation to overcome the significant challenge of delivery across the BBB [114]. A semi-targeted approach is the use of surfactants that can mediate BBB passage as coatings for nanoparticles used in detection of and delivery to brain metastases [115]. Physical disruption, as with focused

ultrasound, is an alternative to biochemical mediators [116]. As immune cells such as macrophages and stem cells have been found to exhibit homing to tumor cells, their use as targeted carriers loaded with therapeutic molecules or nanotherapeutics is an area of active interest [117, 118].

Metastasis to the Liver: Liver (hepatic) metastases are observed with a variety of with cancers but are particularly common with colorectal cancer. Infusion via the hepatic artery is frequently used to achieve regional delivery of chemotherapeutics to the liver, thereby increasing the therapeutic index [119]. To improve further the hepatic distribution, chemotherapeutic drugs have been loaded into beads for controlled release, most notably in the DEBIRI (drug eluting beads loaded with irinotecan) protocol, which has been used in a number of European and U.S. clinical trials [120, 121] and is CE approved. Targeted delivery strategies are also under investigation for reaching hepatic metastases, including folic acid targeting of RNA nanoparticles and mannosylated liposomes that target the non-parenchymal cells of the liver [122, 123].

Metastasis to Bone: Drug delivery to bone has been accomplished frequently in the context of biomaterials serving as bone grafts while delivering therapeutics; this approach is suitable for established bone neoplasms [124]. The fenestrated architecture of bone may allow penetration of nanoparticles with an extended circulation time, as has been shown, for example, in antisense delivery with PLGA particles [125]. The most common strategy for targeting to bone with systemic carriers relies on bisphosphonates, such as alendronate, that localize to the mineralized regions associated with bone neoplasms. Alendronate is readily functionalized to a variety of polymers and liposomes, and its activity assayed via hydroxyapatite binding assay [126-128]. An

interesting approach to increasing the specificity of targeting is to attach the therapeutic to the carrier via a linkage susceptible to proteases such as cathepsin K that are highly expressed at bone resorption sites and in neoplasm [129].

5. "Targeted Imaging" of Metastases

A prerequisite for treatment of metastatic disease is its early identification. The detection of metastases is particularly challenging given their distance from the original disease site, initial small size, and potentially limited contrast relative to its surrounding environment. The targeting approaches being developed to improve the therapeutic index for delivery to metastases are also being applied to develop imaging agents that can be addressed to metastatic lesions. Nanotheranostic strategies where agents are used for detection and treatment are also emerging [130].

A standard-of-care imaging method for the detection of cancers is magnetic resonance imaging (MRI). Nanoformulations incorporating gadolinium for MRI contrast and displaying antibodies or other ligands have been designed to detect metastases in a variety of organs [131]. Surfactant coatings have extended this approach to imaging of brain metastases [115, 132]. Multimodal imaging where MRI is integrated with another imaging method has been demonstrated by utilizing the absorption properties of iron oxide nanoparticles for fluorescence molecular tomography [133]. Positron emission tomography (PET) tracers have been directed to metastatic lesions by conjugation to antibodies for surface markers associated with metastases, such as anti-carcinoembryonic antigen for tracking hepatic metastases of colorectal cancer [134]. Multimodal PET/MRI agents have also been developed using "porphysomes" and directed to bone metastases [135]. Development of a molecularly targeted approach to

imaging a malignant lesion leads naturally to theranostic approaches, such as imaging followed by photothermal ablation of metastases traveling through the lymph [136].

Optical imaging of cellular processes associated with metastasis is widely implemented in the laboratory, yet optical imaging of cancer and in particular of metastasis is less developed. Non-invasive molecular imaging using fluorescent probes [137] and multi-photon microscopy [138] have provided us with greater insights beyond the cellular dynamics of tumor progression such as tumor growth [139], macrometastasis [140], and tumor angiogenesis [141], and have enabled functional readouts of subcellular biological processes such as protein–protein interactions [142, 143]. While optical imaging using fluorescent proteins has helped to advance the study of cancer dynamics in situ, drawbacks relating to interference with tissue absorption and auto-fluorescence leading to low sensitivity of detection of exogenously labeled cells continue to limit adequate cancer resolution in vivo.

The use of near-infrared (NIR) dyes such as quantum dots (QDs) reduces the extent of tissue absorption; however, interference from tissue components and passivation and targeting of QDs remain formidable challenges. An alternative may be the development of "upconversion" fluorophores that absorb light in the NIR and emit in the visible range [144], though in this case attenuation of the emitted signal remains an issue. Recently, fluorophores that are bright, stable, tunable and biocompatible, and emit in the short wave infrared (SWIR) region, which overlaps with the "second and third optical windows" from 1000-1600 nm, have been advanced to overcome the issues of tissue absorption and interference from autofluorescence. Specifically, $NaYF_4$ doped with Yb and Er can be produced as nanocrystals whose emission properties are tunable

via size control. These highly luminescent rare-earth nanomaterials offer superior detection sensitivity over other SWIR emitters while offering the capability of multispectral in vivo SWIR imaging [145]. Human serum albumin encapsulated rare-earth (RE) nanoparticles were used to detect emerging and disseminated tumors in melanoma mouse models [146].

A major impediment to capitalizing on the power of new optical imaging fluorophores is the lack of sufficient contrast between the diseased lesion and healthy tissue. Albumin nanoshells provide a facile platform as targeting ligands can be either adsorbed directly on via the drug binding pockets inherent to albumin or by chemical bioconjugation. Both methods have been utilized for improved targeting of functionalized rare earth-albumin nanocomposites evaluated first using in vitro models of cancers [147, 148] and more recently to identify microscale ($\sim$25 mm^3) metastases in the lungs of mice [149]. These emerging molecular imaging approaches may soon provide avenues to directly visualize biological processes that promote tumor metastasis in a clinical setting. These and similar molecularly tailored imaging technologies [150] could elucidate the dynamics of metastases and profile the molecular expression of metastatic cells, thus illuminating more precise therapeutic targets, which will be an integral tool to personalize therapeutic interventions early in the clinic especially for the hard-to-treat cancers.

6.Perspectives

Metastases are responsible for a major portion of the morbidity and mortality of cancer. Modern research tools have helped us to characterize cancer stem cells as a distinct phenotype within tumors, to understand the physical processes that permit

extravasation, circulation and invasion of colonizing cells, and to compare comprehensively at the genomic and proteomic level the alterations occurring in metastatic cells and heterogeneity across tumors and metastases. In parallel, the potency and specificity of probes for imaging and for therapy continues to improve. By exploiting previously unexplored wavelength ranges and capturing nanoscale electronic transitions, optical imaging probes of tremendous brightness and with the capability of spectral tuning are emerging. Additionally, antibodies, peptides and other ligands can be "evolved" to have very high specificity for a target identified as playing a role in metastatic lesions or in the metastatic process. These have been incorporated in many cases into multifunctional nanoformulations of increasing complexity. These biomimetic approaches may be supplemented with more organic, biologically derived strategies involving tumor exosomes [151] or cells possessing innate homing capacity for tumors and metastases.

Even with high affinity imaging probes and therapeutic antagonists, there remains the enduring challenge of how to target these agents to metastases in such a way that they arrive in sufficient density to provide imaging contrast or pharmacological effect, while maintaining the specificity to distinguish diseased from host tissue. Strategies for targeting agents to metastases can fall into three categories: target the CSCs that give rise to metastases before they occur, disrupt one or more of the mechanistic steps required for metastasis to occur, or if necessary target the nascent tumor in its new microenvironment. The key challenge with any of these approaches remains specificity and potency. By its nature, a metastatic lesion consists of a small number of cells that have evolved resistance mechanisms to conventional

chemotherapy and that is in a location distant from the original site. As such, targeting probes that need to seek out the metastases from the 37 trillion cells in the human body poses a major challenge, analogous to the classic "needle in a haystack" problem.

The challenges discussed above notwithstanding, substantial progress has been made to identify metastases, via the application of a combination of integrative physical and molecular recognition approaches. In addition to the leaky vasculature associated with more established tumors, size-dependent filtering occurs in a variety of organs, including the fenestrated endothelium of liver and spleen, capillaries of bone, and the alveolar space of lungs. These physical effects can be potentially combined with one or more ligand-directed events to create a vehicle that directs imaging probes and modern therapeutics to the nascent metastasis with high levels of precision. Translating the many promising preclinical examples into clinical practice will require continued convergence of precision technologies, advances in molecular biology, and a greater systems-level understanding of the tumor heterogeneity.

Acknowledgements

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