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# Targeting tumor metastases: Drug delivery mechanisms and technologies

Vidya Ganapathy [a], Prabhas V. Moghe [a,b], Charles M. Roth [a,b,*]

[a] Department of Biomedical Engineering, Rutgers, The State University of New Jersey, USA
[b] Department of Chemical and Biochemical Engineering, Rutgers, The State University of New Jersey, USA

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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

* Corresponding author at: 599 Taylor Road, Piscataway, NJ 08854, USA.
*E-mail address:* cmroth@rutgers.edu (C.M. Roth).

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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 (Fig. 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.

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 through 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

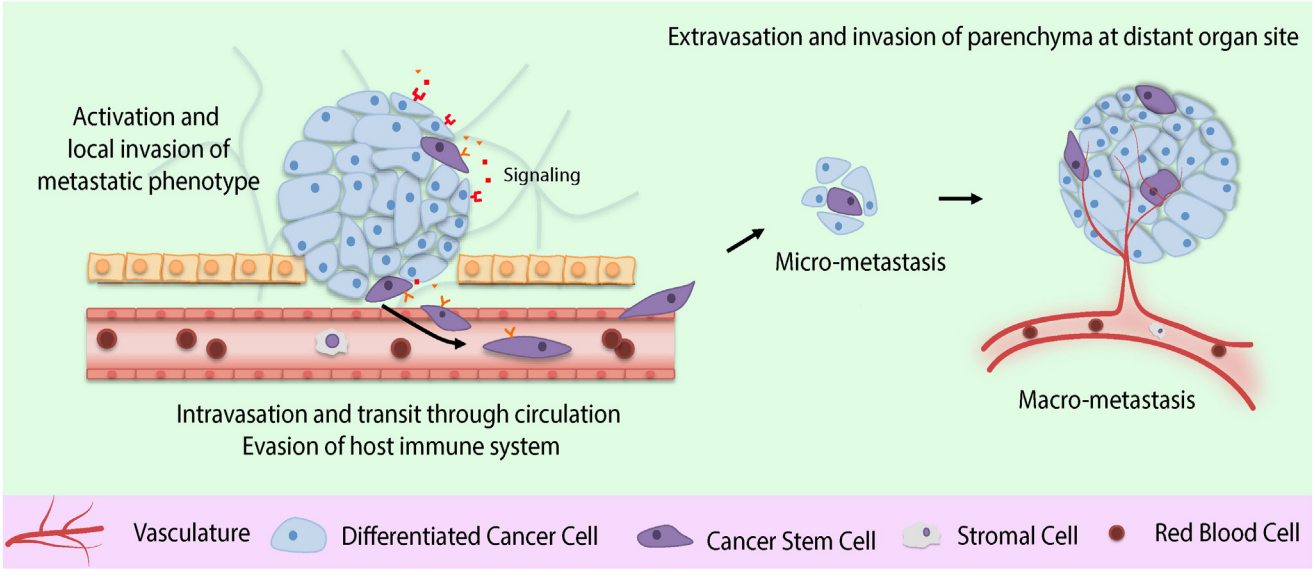


**Fig. 1.** The metastatic cascade — from primary tumor to macrometastases. Beginning with tumor initiating (stem) cells, a stepwise progression occurs from the tumors exiting the primary site of growth (local invasion and activation of metastatic phenotype) followed by systemic translocation (intravasation 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.

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