The Initial Hours of Metastasis: The Importance of Cooperative Host–Tumor Cell Interactions during Hematogenous Dissemination

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ABSTRACT
Tumor cells transit from the primary tumor via the blood circulation to form metastases in distant organs. During this process, tumor cells encounter a number of environmental challenges and stimuli that profoundly impact their metastatic potential. Here, we review the cooperative and dynamic host–tumor cell interactions that support and promote the hematogenous dissemination of cancer cells to sites of distant metastasis. In particular, we discuss what is known about the cross-talk occurring among tumor cells, platelets, leukocytes, and endothelial cells and how these cell–cell interactions are organized both temporally and spatially at sites of extravasation and in the early metastatic niche.

Significance: Metastasis is a function not only of tumor cells but also involves cooperative interactions of those cells with normal cells of the body, in particular platelets and leukocytes. These other cell types alter the behavior of the tumor cells themselves and of endothelial cells lining the vasculature and assist in tumor cell arrest and extravasation at sites of metastasis and subsequently in the establishment of tumor cells in the early metastatic niche. A better understanding of the important role that these contact and paracrine interactions play during metastasis will offer new opportunities for therapeutic intervention.

INTRODUCTION
Metastasis is the cause of about 90% of cancer-associated deaths, yet the mechanisms governing this clinically important process remain poorly understood. Tumor cells can metastasize via the lymphatics to neighboring lymph nodes. However, it remains unclear, in the general case, whether lymph nodes serve as a “way-station” en route to the vasculature. Distant metastases rely on hematogenous dissemination via the blood circulation, and we will concentrate here on this latter process. To metastasize successfully, cancer cells must complete several complex sequential steps: detachment from the primary tumor, intravasation into the vascular system (whether directly or via lymphatics and lymph nodes), survival while in transit through the circulation, initial arrest, extravasation, initial seeding, and survival and proliferation in the target tissue. Despite the fact that large primary tumors can shed millions of cells into the vasculature every day, very few metastases eventually develop (1, 2). Thus, metastasis is, overall, an inefficient process, implying that tumor cells frequently fail to execute one or more of the required steps of the metastatic cascade. Tumor cells that succeed in forming metastases may have acquired the necessary traits to complete these steps while still in the primary tumor, either autonomously or as a result of changes induced by inflammation, stromal cells, or other environmental conditions (e.g., hypoxia and mechanical forces) present in the primary tumor (3). However, the metastatic potential of tumor cells is also further very significantly modulated by the environmental conditions and host cells, in particular platelets and bone marrow–derived cells (BMDC) that tumor cells encounter during their transit through the bloodstream and at the sites of distant metastases. This aspect of the metastatic cascade remains poorly understood because of the technical challenges associated with imaging, isolation, and analysis of circulating tumor cells (CTC) or single disseminated tumor cells that have metastasized to distant organs.
Nevertheless, recent studies using experimental mouse models have begun to show the importance of host–tumor cell interactions, both in the circulation and at sites of extravasation, for the establishment of metastasis. Many of these studies have been conducted with intravenous injections of tumor cells (experimental metastasis), which is generally considered a standard model for studying hematogenous dissemination. Although this experimental setup presents some limitations (e.g., absence of a primary tumor and injection of tumor cells in a single event rather than scattered over a long period of time), it also offers important experimental advantages. It allows close temporal monitoring of the early interactions between single tumor cells and the host microenvironment and a precise characterization of the specific steps of the metastatic cascade affected by a given experimental treatment (4).

In this review, we discuss the sequence of events and key host cell types that interact with tumor cells during their hematogenous transit and their initial establishment at the secondary site and how these interactions influence metastasis and cancer prognosis.

Transit through the Bloodstream and Initial Arrest (First Minutes)

CTCs are frequently found in the blood of patients with primary solid tumors, and it is generally assumed that a subset of these cells will eventually give rise to distant metastases (5, 6). However, as indicated by intravenous injection of tumor cells into animal models, CTCs typically do not spend much time circulating through the bloodstream. Indeed, most carcinoma cells have diameters that are too large to pass through small capillaries and many are, therefore, trapped in the first capillary bed that they encounter within minutes of entering the circulation (Fig. 1; ref. 2). During this short period of transit, as well as during initial arrest, cells remain exposed to the blood flow and are vulnerable to death induced by shear stress and turbulence or by immune cells, particularly neutrophils (Fig. 2A; ref. 2). By that time, most tumor cells have exited the bloodstream and seeded into the stroma of the secondary site and additional myeloid cells are recruited to this initial metastatic niche. The tumor cells may reinitiate growth to form metastases within a few weeks. Alternatively, tumor cells can survive and stay dormant for a long period before reinitiating growth and thus form clinically relevant metastases only months or years later. Overall, only a few cells successfully complete the metastatic cascade and give rise to overt metastases. So far, most studies of host–tumor cell interactions during metastasis have been conducted at single time points arbitrarily chosen by investigators, whereas only a few studies have examined the temporal recruitment of host cells by real-time imaging or sampling at multiple time points. This scheme is thus a tentative summary of many independent studies reporting experimental observations at different time points and obtained with different model systems.

In this respect, activation of the coagulation cascade and the formation of platelet-rich thrombi around tumor cells in the vasculature have both been proposed to play major roles in physically shielding CTCs from the stress of blood flow and from lysis by NK cells (Fig. 2A; refs. 7–11). Tissue factor expressed by tumor cells triggers the formation of thrombin, which leads to both coagulation and platelet activation. In turn, coagulation and platelet activation enhance metastatic spread (11–16). Fibrin can be bound by integrins, αvβ3 on tumor cells and αIIbβ3 on activated platelets, leading to formation of tumor cell–fibrin–platelet aggregates. Therefore, genetic elimination or blockade of β3 integrins on platelets or tumor cells compromises metastasis (8–10, 17–19). Furthermore, expression of diverse P-selectin ligands by tumor cells also favors their interaction with P-selectin on the surfaces of activated platelets (20). Consequently, platelet–tumor cell microthrombi do not form in P-selectin−/− mice and these mice are protected from metastasis (21, 22). More generally, diverse intact platelet functions (e.g., platelet activation, adhesion via surface receptors, and TGF-β release) have been shown to be necessary for efficient metastasis in many experimental models (10, 17, 18, 23–26). Platelets are in fact the major source of TGF-β in the circulation (23). Consistent with a prometastatic role of platelets, high platelet counts and coagulation correlate with decreased patient survival in many different types of cancers, although some of that correlation could well be because of thrombosis caused by the presence of a tumor (9, 10, 27, 28).

In addition to providing physical shielding, platelets and coagulation have been shown to impair NK cell tumorolytic activity in vitro (7, 8). For example, platelet-derived TGF-β downregulates the activating immunoreceptor NKG2D on NK cells (29) and platelet-derived growth factor (PDGF) released by platelets can also suppress NK cell function (30). The coating of the surfaces of tumor cells with normal
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platelet-derived MHC class I may also favor tumor cell escape from the innate immune system (31). Thus, multiple platelet–tumor cell interactions may lead to the inhibition of NK cells, leading to increased tumor cell survival in the circulation.

Clustering of tumor cells and adhesion with other cell types have also been proposed to contribute to successful tumor cell survival in the circulation. For example, CTC clusters isolated from the blood of patients with metastatic prostate cancer have higher hematoxylin and eosin staining intensity than do individual CTCs, suggesting reduced cell death and potential protection from shear stress (6, 32). Similarly, CTCs incorporated in heterotypic tumor–fibroblast...
aggregates retrieved from the blood of tumor-bearing mice have improved viability compared with single CTCs (33). Given the enhancing effects of platelets on metastasis, it is plausible that the CTCs that are most effective in metastasis will prove to be those in aggregates with platelets and possibly also leukocytes. Most current methods for scoring CTCs in patients score only single cells and could be missing an important fraction of the CTC population.

** Arrest and Adhesion to the Vascular Wall (First Hours)**

The propensity of tumor cells to metastasize to specific organs is in part dependent on the circulation pattern, and the preferred sites of metastasis for a given type of cancer often include the first capillary beds downstream of the primary tumor. Examples are metastasis of colon cancer cells to the liver and of breast cancer cells to the lungs, where the initial arrest of tumor cells may be mainly caused by physical restriction in capillaries of small diameter (2). In such cases, the formation of aggregates comprising CTCs and host cells may enhance passive trapping in capillaries by increasing the diameter of tumor cell emboli. However, during metastasis to either the liver or the lung, tumor cells can also arrest in vessels of larger diameter than capillaries (34), showing that active adhesion to the vasculature via specific proteins, such as selectins, integrins, and metalloprotease, can also contribute to initial arrest (19, 35–38). Importantly, some of these adhesion receptors could be contributed by associated platelets, leukocytes, or stromal fibroblasts.

It is likely that initial trapping, which occurs within minutes of the entry of tumor cells into the circulation, is mostly passive and dependent on circulation patterns, whereas the cells that permanently arrest are those that form specific, longer-lasting adhesive interactions with the endothelium. In accordance with this concept, a high proportion of tumor cells rapidly arrests in capillaries in experimental metastasis models, whereas sustained adhesion to the endothelium leading to permanent seeding seems to be of variable efficiency and often fails. Indeed, whereas some studies have shown that more than 80% of tumor cells survive the circulatory phase of metastasis (44). Soluble factors secreted by primary tumors increase the formation of hyperpermeable foci via localized activation of endothelial focal adhesion kinase and E-selectin, which in turn favors the adhesion of tumor cells to the endothelium (44). In addition, soluble factors secreted by primary tumors have been reported to induce the recruitment of BMDCs to specific areas of distant organs to form so-called premetastatic niches. These niches have been proposed to create a supportive environment for the survival and growth of incoming tumor cells (45–49). The presence of a primary tumor also triggers inflammation, which leads to the activation of the endothelium and platelets and contributes to the systemic mobilization of various types of BMDCs (immature myeloid cells, neutrophils, and monocytes), which may all play critical and concerted roles in metastasis (3, 42, 49–51).

The presence of an activated endothelium may favor arrest and adhesion of tumor cells and this likely involves participation of myeloid cells (Fig. 2B). For example, activation of the endothelium by interleukin (IL)-1α, IL-1β, or TNF-α leads to the expression of E-selectin and P-selectin as well as vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 at the surfaces of endothelial cells. Binding of these cell adhesion molecules to their ligands on tumor cells can then promote tumor cell rolling and adhesion (20, 52, 53). Interestingly, in a liver metastasis model, the presence of tumor cells triggers the production of TNF-α by Kupffer cells, showing that immune cells can play an active part in endothelial cell activation and, therefore, in favoring metastatic arrest (54). Similarly, Laubli and colleagues (55) showed that activation of the endothelium in vitro by tumor cells is P-selectin dependent and requires the simultaneous presence of platelets and neutrophils together with tumor cells. In addition to favoring tumor cell adhesion, the activated endothelium secretes the inflammatory cytokine CCL5, which promotes the recruitment of monocytes in proximity to the tumor cells. Importantly, platelets also secrete high levels of a plethora of growth factors and cytokines (e.g., PDGF, TGF-β, PF4/CXCL4, VEGF, stromal cell-derived factor (SDF)-1/CXCL12, CXCL7, and CCL5), which could also contribute to endothelial activation or directly lead to the recruitment of BMDCs (56). Furthermore, the presence of P-selectin on activated platelets adherent to the endothelium enhances the recruitment of leukocytes via binding to P-selectin glycoprotein ligand-1 on leukocytes (57–59). This interaction promotes the activation of the leukocyte β2 integrins, which then bind to fibrinogen presented by αIIbβ3 integrin and to glycoprotein (GP)IIb/IIIa, ICAM-2, and junctional adhesion molecule (JAM)-3, all present on the surfaces of platelets, and thereby stabilizes platelet-leukocyte interactions (60–63). Thus, the formation of cellular assemblies composed of tumor cells, platelets, leukocytes, and activated endothelium appear very likely to be required for efficient metastasis.

Although the contributions of leukocytes to the primary tumor are well established, their roles in the processes of metastasis have been less well characterized until recently. Globally, leukocytes have been shown to support the early stages of metastasis, as illustrated by the decrease in leukocyte–tumor cell interactions and impaired early tumor cell seeding in L-selectin−/− mice (64). Similarly, metastasis was attenuated in mice unable to induce L-selectin ligand expression at sites of intravascular tumor cell arrest (40). Moreover, metastasis is
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reduced by genetic or pharmacologic ablation of monocyte/macrophage-lineage cells (42, 51, 65), and tail-vein injection of neutrophils 1 hour after injection of melanoma cells results in increased retention of tumor cells in the lungs after 24 hours (ref. 66; Fig. 1, 2B). These latter observations may be explained by the secretion of IL-8 by tumor cells, which can attract myeloid cells to the vicinity of tumor cells (65). The activated endothelial cells secrete CCL5, which also enhances coagulation, and this too contributes to attract myeloid cells to the vicinity of tumor cells (65). The monocyte/macrophage-lineage cells recruited by the tumor cells were shown to enhance the seeding of metastatic mammary tumor cells in the lung (42, 51, 65). Among these cell populations, a distinct set of metastasis-associated macrophages (F4/80+CD11b+Gr1−) secrete VEGF-A that promotes the extravasation, seeding, and growth of the tumor cells (42), presumably via increased endothelial permeability. However, in a colon carcinoma experimental metastasis model, tumor cell–derived CCL2 has also been shown to signal directly to CCR2 expressed by endothelial cells, resulting in an increase in vascular permeability and subsequent metastasis by a mechanism independent of myeloid cells (Fig. 2C; ref. 75). Thus, tumor cell extravasation is facilitated by multiple complex interactive networks comprising direct platelet-to-tumor cell signaling, tumor cell-to-endothelium signaling, and monocyte/macrophage-to-endothelium signaling.

Another example of a prometastatic cascade of events involving multiple types of host cells was provided by Laubli and colleagues (55), who showed that colon carcinoma cells, together with platelets and neutrophils, activate the endothelium. In turn, the activated endothelial cells secrete CCL5, which leads to increased recruitment of monocytes to the tumor cells. In this model, monocyte recruitment occurs after 2 days (55), a time point at which platelets are no longer associated with tumor cells, illustrating the sequential involvement of different host cells in supporting metastatic seeding. Although not yet tested, it is a plausible hypothesis that early and transient platelet–tumor cell interactions trigger a cascade of paracrine signals impinging on the recruitment and function of various types of leukocytes, which in turn contribute to survival and metastasis of cancer cells. Indeed, macrophages and specific subsets of bone marrow–derived immature cells have been implicated in promoting cell survival and proliferation in models of metastasis to the lungs. For example, binding of VCAM-1 aberrantly expressed by tumor cells to α4 integrin expressed by macrophages, protects cancer cells from proapoptotic cytokines such as TRAIL, leading to increased survival and metastasis (76). Other examples of the importance of tumor cell–stroma interactions for early metastatic colonization come from recent studies, which showed requirements for peristostin and tenascin C expression by fibroblasts at the site of metastasis for successful
metastatic growth (Fig. 2C; refs. 77, 78). TGF-β seems to be involved in the enhancement of the expression of these ECM proteins, suggesting that TGF-β expressed by tumor cells or host cells (such as platelets, as discussed above) may be important for the initiation of this supportive metastatic niche (78–80). Finally, it is likely that the tumor-promoting effects of BMDCs, which are increasingly well understood for tumor progression at the primary site, may also be important for the subsequent establishment of overt metastases.

**The First Hours of Metastasis as a Possible Therapeutic Target**

Most of the approved anticancer therapies inhibit the growth of primary tumors. Although some of those therapies also have an effect on metastatic growth, there are currently no therapies specifically aimed at preventing the metastatic process by targeting the different steps of the metastatic cascade. Furthermore, although some potential antimetastatic compounds have been identified in preclinical models, there is a clear need for clinical trials specifically designed to test for antimitastatic effects (e.g., time required for the formation of a new metastasis) rather than for the ability of compounds to prevent tumor growth (81).

The early steps of the metastatic cascade discussed in this review are generally not considered as attractive clinical targets. The rationale for this opinion is that tumor cells can disseminate early during tumor progression (82, 83), and therefore it is likely that some metastatic cancer cells have already completed the early steps of the metastatic cascade by the time of cancer diagnosis. Thus, the later steps comprising escape from dormancy, reinitiation of growth, colonization, and survival in the metastatic niche are likely better targets for therapeutic intervention. Indeed, although CTCs can complete the steps of the metastatic cascade leading to seeding within a few days, reinitiation of growth can be significantly delayed and metastatic growth occurs over an extended period of time, providing a more manageable time window for therapeutic intervention.

That said, the early steps of metastatic dissemination discussed here may offer some new opportunities for therapeutic interventions targeting molecular mechanisms and cellular processes such as adhesion, migration, invasion, and epithelial–mesenchymal transition, that are not affected by the cytotoxic or antiproliferative effects of most traditional anticancer therapies. In addition, cells transiting through the bloodstream may be particularly accessible to pharmacologic intervention. Drugs or combinations of drugs impairing not only the ability of tumor cells to proliferate, but also their ability to interact with host cells and complete the early steps of the metastatic cascade, may prove beneficial to prevent further metastatic dissemination either from the primary tumor or from already existing metastases. Indeed, it has been shown in animal models that tumor cells from metastases have the ability to reenter the circulation and seed other metastases (84, 85) or to self-seed back at the primary tumor site, further contributing to cancer progression (86). Thus, inhibitors of metastatic arrest, extravasation, or seeding may impact overall disease progression, even if disseminated tumor cells are already present in a patient. However, the patients most likely to benefit from metastatic prevention therapy would be those who have been diagnosed at early stages before detectable metastatic spread, or even people that do not have the disease but are at high risk for developing highly metastatic cancers.

Specific inhibitors of metastasis could also be envisaged for cases in which surgical ablation of the primary tumor is not possible, or during the perioperative period, as surgery may enhance the release of CTCs into the bloodstream (87, 88).

Even though many aspects of the early steps of metastasis are still incompletely understood, molecules that are critical for the completion of specific steps of the metastatic cascade are starting to emerge and could possibly be exploited as therapeutic targets. Inhibitors of a few of the signaling pathways involved in the early steps of metastasis (e.g., VEGF-A, TGF-β, NF-κB, and CCL2) are already used in the clinic or are being evaluated in clinical trials for the treatment of cancers or other diseases. Furthermore, interfering with the ability of tumor cells to recruit or interact with supportive host cells may prevent the formation of optimal conditions for metastatic progression. In this respect, inhibitors of cell adhesion receptors required for the tumor cell–host cell interactions may be of particular interest.

For example, the anticoagulants heparin or low-molecular-weight heparin (LMWH) have already been shown to prevent metastasis in preclinical models by inhibiting the formation of platelet-tumor cell aggregates (22). More importantly, a number of independent clinical trials have shown that treatment with LMWH improves the survival of cancer patients (9, 89–91). Interestingly, the beneficial effects of a LMWH treatment were predominately seen in patients with good prognosis or that did not have detectable metastasis at the onset of treatment, consistent with a role for LMWH in inhibiting the seeding of metastases rather than in the growth of existing ones (9, 89–91). Mechanistically, the effect of heparin on metastasis is attributed primarily to its ability to inhibit the interaction of P-selectin with its ligands and not to its anticoagulant activity. Indeed, the synthetic LMWH fondaparinux, which does not inhibit P-selectin but retains anticoagulant activity, fails to inhibit experimental metastasis (92).

In addition to inhibiting P-selectin, heparin can also inhibit L-selectin and α4β1 and αIIbβ3 integrins (93), providing indications that heparin might interfere with multiple prometastatic adhesive interactions. Similarly, function-blocking antibodies targeting α4β1 or αIIbβ3 integrins inhibit experimental metastasis (94, 95). Moreover, small molecules and antibodies that inhibit the function of αIIbβ3 integrin or the binding of VCAM-1 to α4 integrin are already available in the clinic. Antagonists of αIIbβ3 integrin are used as anti-thrombotics and could be expected to block platelet-tumor cell interactions through fibrinogen as would also be true for antagonists of α5β integrin, present on many tumor cells. Antagonists of α4 and β2 integrins have been developed for the treatment of diseases involving influx of leukocytes, such as inflammation and autoimmunity, and might thus also interfere with the macrophage–tumor cell interactions that promote tumor cell arrest, survival, and reinitiation of tumor growth at the site of metastasis (96, 97).

Overall, although inhibitors of host–tumor cell signaling interactions show promise in experimental models, it remains to be tested whether these agents will prevent or significantly delay metastasis in cancer patients, and clinical
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trials will be challenging, given the long time scales necessary. Furthermore, potential side effects affecting vital functions, such as the immune response, coagulation, and hemostasis, will need to be carefully evaluated. Thus, a better and more comprehensive understanding of the molecular mechanisms involved in metastasis is required for the development of specific therapies with minimal potential adverse effects and efficient blocking of cancer metastasis.

CONCLUSIONS

Although the complexity of the metastatic cascade has been acknowledged for many years, the active participation of cells of the host microenvironment to metastatic dissemination is only beginning to be appreciated. The studies reviewed herein provide examples of the importance of dynamic tumor-host cell interactions at each step of the metastatic cascade (Figs. 1 and 2). The context-dependent and concerted actions of different populations of host cells appear to be necessary for efficient metastasis. However, exactly how the different types of host cells interact with each other as well as with tumor cells both temporally and spatially, and the precise hierarchy and function of these interactions, remain incompletely understood. Answering these fundamental questions will likely provide important clues not only about the molecular mechanisms involved during metastatic dissemination but also about how these early processes influence the subsequent metastatic colonization. Deeper understanding of these diverse tumor-host cell interactions may also offer possibilities for novel therapeutic interventions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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