Molecular events of brain metastasis

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✓The brain is a privileged site of systemic cancer metastasis. The stages of the metastatic journey from the periphery to the brain are driven by molecular events that tie the original site of disease to the distant host tissue. This preference is not arbitrary but rather a directed phenomenon that includes such critical steps as angiogenesis and the preparation of the premetastatic niche. It appears that the connection between naïve brain and cancer cells is made in advance of any metastatic breach of the blood–brain barrier. This contributes to the preferential homing of cancer cells to the brain. Delineation of the guidance mechanisms and elements that influence cancer cell motility and dormancy are important for the advancement of treatment modalities aimed at the remediation of this devastating disease.

KEY WORDS • brain metastasis • homing • angiogenesis • premetastatic niche • epithelial–mesenchymal transition

METASTASIS to the brain is a frequent complication of many systemic cancers, with a yearly incidence in the US of approximately 170,000, occurring in approximately one third of all cancer patients.^{14,39} Brain metastasis represents the most common form of intracranial cancer, appearing 10 times more often than primary brain tumors.² The incidence of metastasis to the brain appears to have recently increased, possibly due to the induction of more aggressive clinical management, extending survival.⁴⁶ Moreover, the detection of metastatic lesions to the brain has also improved, as we are now able to visualize smaller foci by using advanced imaging methods.⁵³

In adults, the most common sources of metastatic lesions to the brain include the lung (50–60%), breast (15–20%), skin (5–10%), and gastrointestinal tract (4–6%).^{1,32,33,38,52} Systemic cancers in patients who present with brain metastasis can progress at different rates. For example, 10 to 14% of patients will harbor detectable brain metastasis on the initial diagnosis of small cell lung cancer.³⁸ This factor highlights the short interval between detection of primary lung malignancy and the manifestation of brain metastasis. In contrast, the appearance of brain metastases from a primary breast cancer occurs at a later stage of the disease process, indicating a prolonged

dormancy.^{15,16} Another critical distinguishing feature is the number of brain metastases different cancer types manifest. Briefly, patients with breast cancer are likely to have multiple intracerebral metastases: 78% have two or more metastatic lesions.¹⁰

In this paper, we address the key events that lead to macrometastasis in an attempt to further explain some of the aforementioned epiphenomena, such as dormancy and multiplicity. We also offer a paradigm for the preferential homing of metastatic cells to the brain.

MECHANISM OVERVIEW

The net growth of a solid tumor is a result of the delicate balance between proliferation and apoptosis of its cell population. As the tumor expands, a critical time period is reached when signals for invasion of the host tissue are elaborated. Invading cells detach from the tumor mass, disperse and traverse the epithelial/endothelial boundary, and then use the vasculature as a conduit for the colonization of distant organs. Within the vasculature, cancer cells evade immune surveillance to ensure survival. Reattachment to and disruption of the endothelial barrier leads to establishment of distant micrometastasis and dormancy. The expansion of tumor cells into a macrometastasis is a process that requires modification within the host tissue, including rearrangement of the extracellular matrix, recruitment of cells into the developing stroma, and neovascularization. Each of these steps is the result of a complex process integrating extracellular and intracellular signals that secure the survival of a select population of cancer cells.

Abbreviations used in this paper: CNS = central nervous system; FAK = focal adhesion kinase; GBM = glioblastoma multiforme; MMP = matrix metalloproteinase; TNF α = tumor necrosis factor- α ; VEGF = vascular endothelial growth factor.

MOBILIZATION PROCESS

The ability of cancer cells to invade surrounding tissue while ignoring physiological boundaries is a prerequisite for malignant metastasis. It requires an alteration in the interaction of and adherence between the cancer and surrounding tissue cells. Once it reaches the size of 1 to 2 cm in diameter, a primary tumor must promote angiogenesis to coopt neovascular infiltration as a means of in situ survival. Factors are amplified—most notably VEGF in the setting of relative hypoxia among oxygen-starved overgrown tumor clusters—to coopt native blood vessels toward paving new capillary networks initially at the primary site and later at the site of metastasis.¹⁰ Continued growth of the primary tumor heightens the risk of ultimate spread to the brain and other organs but is not necessary: brain metastases have been known to cause death from undetected primary tumors. In general, however, the larger the tumor at the primary site becomes and the greater the degree of neovascularization, the stronger the likelihood of spillage into the systemic vasculature and diffuse migration. Angiogenesis by itself at the site of primary tumor does not guarantee metastasis. Tumor cells should also be able to invade through basement membranes and escape immune detection with the aid of MMPs and through the loss of surface adhesion molecules, respectively.^{17,47} A change in the expression of integrin and adhesion molecules in cancer cells seems to signal the initiation of invasion. Cadherins play an important role in cell-to-cell interaction. It has been shown that modulation of E-cadherin to N-cadherin acts as an on switch for the cancer cells to invade. Whereas E-cadherin promotes cell adherence, N-cadherin promotes cell mobility.²⁴

Chemokines also play a pivotal role in the dynamic process of mobilization.⁹ They recruit inflammatory cells to the leading edge of the tumor, and they trigger inflammatory cell protease release to aid in the preparation of the stroma. Well-known proteases such as MMPs, plasmin, urokinase plasminogen activator, cathepsins, and heparinases work in concert to disintegrate the extracellular matrix and cause the release of embedded growth factors like VEGF. Collectively, they form the medium for invasion and migration.

The mobilization of cancer cells initiates invasion and metastasis. Generally, the leading edge of migrating cells is marked by various cell protrusions (lamellipodia) and focal adhesions (podosomes).¹³ Both of these organelles are actively engaged in matrix remodeling and tissue invasion. Free-floating growth factors within the extracellular matrix bind to receptor tyrosine kinase at the leading edge of the migrating cells (Fig. 1). Receptor tyrosine kinase interacts with integrins to form an FAK-Src complex triggering the formation of lamellipodia.³ The other function of the FAK complex is to activate guanosine triphosphatase CD42 and Rac. Guanosine triphosphatase CD42 promotes cytoskeletal contraction and facilitates cellular forward motion, whereas Rac activates MMPs, which help to prepare the advancing path. Additionally, the binding of FAK to p190RhoGEF activates Rho-dependent cytoplasmic actin stress fibers and the maturation/stabilization of focal adhesions at the leading edge of the migrating cells. At the trailing edge, FAK is dephosphorylated by protein tyrosine phosphatase, resulting in the degradation of the

focal adhesion complexes and detachment from the extracellular matrix.²³ On making the epithelial–mesenchymal transition, cancer cells actively seek out the vessels by following chemoattractants emanating from the endothelial cells. They then penetrate blood vessels by breaching the epithelial–endothelial boundary to complete their journey into the circulation. Twist, a master regulator of embryonic morphogenesis, seems to play a pivotal role in this transition.²⁹ This transition is essential to override death on detachment, or “anoikis,” into the circulation, thus improving metastatic efficiency. At the height of tumor angiogenesis, endothelial cells and tumor cells are positioned in proximity to one another, producing so-called vascular mimicry, allowing for easy access of tumor cells to the circulation.

CIRCULATION AND HOMING

Once escape into the systemic circulation has been achieved, migrating tumor cells still encounter significant resistance. Cell survival in the circulation is difficult due to a combination of shear forces, phagocytosis, and obstruction within capillary beds. Only 1.5% of nonhematogenous cells injected into the bloodstream survive for at least a 24-hour period.³⁷ Cells released directly into the pulmonary circulation have a better chance of metastasizing to the brain because of their direct access to the heart and systemic arteries, whereas other tumor types must first survive passage through the capillaries of the pulmonary vasculature.⁵⁵ The significant risk of lung cancer metastasizing to the brain may be partly explained by this relative ease of access.

It has been shown that circulating cancer cells are shielded by platelet aggregates and other components of the coagulation cascade.¹¹ This phenomenon represents a mechanism by which they avoid immunodetection and optimize their ability to overcome shear force. The shielding process may be at the root of the hypercoagulable state seen in systemic cancer. Cancer cells arrest circulation by binding to coagulation factors such as tissue factor, fibrinogen, fibrin, and thrombin. These “cancer thrombi” may trigger the induction of TNF α , resulting in the expression of E- and P-selectins on endothelial cells, and thus facilitating their own arrest.²⁰ The expression of E- and P-selectin on endothelial cells initiates tethering and rolling of the cancer cells and formation of weak adhesions to the endothelial cells within the capillary bed.³¹ Furthermore, emerging evidence suggests that endothelial cells from various organs express tissue-specific surface proteins. Metadherin is one such protein, which mediates homing of tumor cells to the lung preferentially, and may aid in arresting and anchoring cancer cells to the endothelial surface.²⁰ In the case of breast cancer metastasis to the central nervous system, the CD44v isoform has been reported to differentiate metastasis to the brain from that to the spinal cord.²¹ Other surface molecules known to be expressed on cancer cells, such as integrin $\alpha 3 \beta 1$,⁵¹ will bind to laminin-5 on the vascular basement membrane. This binding is thought to be an important step in certain types of metastasis, including that from the lung. Similarly, cancer cells that express CXCR4 have an affinity for CXCL12-rich tissues such as the lung.⁴ The interaction of these surface proteins and cognate ligands and/or

receptors expressed in various host organs may explain the homing process and preferential colonization of cancer cells to host organs.⁴¹ It is conceivable that cancer cells will metastasize to organs from a similar embryological origin. Hence, malignant melanoma has a greater likelihood of forming leptomeningeal metastasis since they are both derived from neural crest cells.³⁰ Recently, it has been shown that embryological development and tumorigenesis use the same transduction pathways. Nodal, a member of the transforming growth factor- β superfamily, is expressed in malignant melanoma cells; when these cells are placed in embryonic tissue they induce ectopic formation of the embryonic axis.⁵⁰ Furthermore, Nodal induces the mobility and transformation of normal melanocytes and embryonic progenitors of their host organs, indicating a bidirectional communication between metastatic cancer cells and their distant host.⁷ This finding concurs with what was described by Paget³⁵ in 1889: “when plant goes to seed, its seeds are carried in all directions, but they can only live and grow if they fall on congenial soil.” In contrast to this dictum, however, tumor metastasis does not follow a random route but is governed tightly by the embryonic development of the cancer cells and their host tissue.

Colonization Process

Once lodged and anchored onto the endothelial surface, cancer cells extravasate into the parenchyma. They make use of the local proinflammatory and hypoxic response of the tissue to gain passage into the brain, breaching the blood–brain barrier with its tightly adjoined endothelial, basal lamina/pericytic, and glial cells. Local extravasations and the aforementioned proinflammatory responses help to set up the premetastatic niche for colonization, including the activation of MMP9.⁴⁵ Emerging evidence has shown that VEGF, produced by the primary tumor, activates bone marrow cells. These cells carry the myeloprogenitor and endothelial progenitor phenotypes and are derived from hematopoietic lineage. They are recruited to the developing premetastatic niche, where they later undergo colonization. They may also participate in preparing the stroma and initiating angiogenesis.⁴⁵ These bone marrow–derived endothelial cells have been shown to contribute in a significant way to neovascularization.

On arrival at the premetastatic niche, cancer cells can subsist in a dormant state,^{19,42} which is mediated by inhibition of neovascularization. The balance of proangiogenic factors (VEGF, platelet-derived growth factor, endothelial growth factor, and so forth) and antiangiogenic factors (thrombospondin-1, platelet factor IV, and so forth) determines the course of disease progression. Removal of inhibitory antiangiogenic factors can lead to the growth of metastases from dormancy.³⁶

The dormant state of metastatic cancer cells is analogous to that of cancer stem cells: once activated, both embody enormous potential for proliferation. Both require a permissive milieu, a premetastatic niche, to attain their proliferation potential. The premetastatic niche shares features similar to epithelial restitution.⁴⁴ This process involves the upregulation of Hedgehog and Wnt pathways. Hedgehog promotes collagen matrix invasion and the expression of genes associated with the epithelial/mesenchymal transition such as *SNAI1* and *SNAI2*. These genes

FIG. 1. Schematic depicting the major events and participating molecules in the process of metastasis: invasion; epithelial–mesenchymal transition; circulation shielding; anchoring and extravasation; dormancy and macrometastasis. ANG = angiopoietin; ECM = extracellular matrix; RTK = receptor tyrosine kinase; TGF = transforming growth factor.

induce aggressive behavior of cancer in colorectal carcinoma.²² Hedgehog is also involved in the maturation and development of the cerebellum. Hence, it may also be responsible for the propensity of colorectal carcinoma to metastasize to the posterior fossa.^{6,43,54}

The inflammatory response is an important element in grooming the premetastatic niche. Reactive monocytosis occurs in response to systemic cancers, primary tumors of the CNS (such as GBM), and metastases to the brain. However, when comparing GBM with brain metastases, it is only the latter that demonstrate a true increase in the absolute numbers of circulating monocytes.⁵ Moreover, amplification of tumor necrosis factor receptor is also unique to brain metastases when compared with GBM.⁷ It is well established that circulating monocytes contribute to the cachectic state that hallmarks the progression of systemic diseases via secretion of TNF α .³⁴ Furthermore, it has recently been shown that TNF secretion by activated microglia of the CNS is capable of inducing systemic tumor cell growth transplanted to the brain.

The preferential tendencies of certain cancer types to metastasize to the CNS have not been fully elucidated. Data from recent studies have shown that heparinase, an endo- β -D-glucuronidase enzyme, is a potent molecular determinant of melanoma metastasis.³⁴ The invasiveness of systemic cancer cells expressing heparinase is attribut-

able to its catalytic breakdown of heparin sulfate chains present on proteoglycans constituting the extracellular and basement membranes of the blood-brain barrier, thus leading to cellular degradation and remodeling.^{3,27} Moreover, after lysis ensues, cleavage products promote propagation, invasion, and initiation of angiogenesis to prepare the permissive environment.^{8,12,25,26,28,48} These cleavage products also augment Akt and PI3K- and p38-dependent endothelial cell migration. Additionally, they activate the Src pathway, resulting in stimulation of VEGF expression and providing angiogenic and survival signals to the endothelial cells in nascent vessels.⁴⁰ The levels of heparinase correlate directly with an upregulation in the aggressive nature of metastatic tumors and inversely with the prognosis of postoperative cancer patients.¹⁸ A significant nexus exists with naïve astrocytes, as they are also capable of producing heparinase. It should be noted that the same isoform of heparinase is found in cancer cells and naïve astrocytes.^{49,50} It is plausible that the brain, even before a neoplasm invades, is able to generate the necessary cytokines in anticipation and preparation of the ensuing metastatic colonization. Directed guidance of melanoma to the brain appears to be facilitated by activation of the same signal both in the periphery and the brain. Moreover, the robust interactions between premetastatic milieu host cells and colonizing cancer cells may play a pivotal role in determining the multiplicity of metastases in the brain.

CONCLUSIONS

The brain has long been recognized as a favored site for metastatic deposition. Although we are beginning to understand tumorigenesis and the spread of systemic cancer, the mechanism responsible for malignant disbursement to the brain remains elusive. In this paper, we have highlighted the current understanding of the metastatic process with an emphasis on the mobilization of cancer cells, the concept of epithelial-mesenchymal transition, and the importance of a premetastatic milieu for distant colonization.

We hypothesize that metastasis to the brain is augmented by the activation of homologous molecules between systemic cancer and brain parenchyma. These molecules, first expressed by the primary cancers, are then echoed in the brain. Using this beacon, cancer cells selectively home-

epithelial-mesenchymal transitions by repressing E-cadherin expression. **Nat Cell Biol** 2:76–83, 2000

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Manuscript submitted December 18, 2006.

Accepted January 29, 2007.

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