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Seed, soil, and beyond: The basic biology of brain metastasis

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Abstract

First invoked by Paget, the seed and soil hypothesis suggests that the successful growth of metastatic cells depends on the interactions and properties of cancer cells (seeds) and their potential target organs (soil). In the context of the seed and soil hypothesis this review examines recent advances in the understanding of molecular and cellular features that permit transformed epithelial cells to gain access to the blood stream (intravasation), survive their journey through the blood stream, and ultimately traverse through the microvasculature of target organs (extravsation) to deposit, survive, and grow in a foreign tissue environment. In addition to a review of the clinical and experimental evidence supporting the seed and soil theory to cancer metastasis, additional concepts highlighted include: (i) The role of cancer stem-like cells as putative cells of metastatic origin (the "seeds"); (ii) the mechanism of epithelial to mesenchymal transition (EMT) in driving epithelial cell conthose molecules do no blood stream to avoid anoikis, or anchorage independent cell death; and (iv) the reverse process of EMT, or mesenchymal to epithelial transition (MET), which promotes conversion back to the parent cell morphology and growth of macrometastsis in the target organ. The unique biology of metastases once established in the brain, and in particular the "sanctuary" role that the brain microenvironment plays in promoting metastatic growth and treatment resistance, will also be examined. These issues are of more than academic interest since as systemic therapies gradually improve local tumor control, the relative impact of brain metastasis will inexorably play a proportionally greater role in determining patient morbidity and mortality.

Key Words: Brain metastasis, cerebral metastasis, EMT, MET, Paget, perivascular niche, seed vs soil

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"The seeds of a plant are carried in all directions; but they can only live and grow if they fall on congenial soil"
– Paget 1889 [59]

INTRODUCTION

In the United States, greater than 40% of cancer patients develop metastasis to the brain.[25,80] Frequently encountered pathologies include lung, breast, melanoma, renal, and colorectal tumors [Table 1].[16,74] Consistent with vascular distribution and tissue volumes 80% of brain metastases occur in the cerebral hemispheres, 15% in the cerebellum, and 5% in the brain stem.[18,67] After diagnosis of metastasis to the brain, the median survival of untreated patients is 1-2 months, extended only to 6 months in patients treated with surgery, chemotherapy,

and radiation.[72] Strong prognostic factors include good functional status, age <65 years, solitary metastasis, controlled primary tumor, long interval from primary diagnosis to brain disease, and the presence of certain cancer subtypes such as HER2 positive breast cancer or EGFR mutant nonsmall cell lung cancer.[20] Therefore, an understanding of the process of metastasis with an eye toward even preventing brain metastasis is critical for improving patient outcomes.

Certainly, the process of metastasis testifies to the hardiness of cancer cells and subsequent resistance to treatment. To successfully metastasize to the brain is a rigorous and complex cellular process. In step-wise fashion, an epithelial derived cancer cell must free itself of cell–cell and cell–basal lamina constraints imposed by the parent epithelial tissue, lyse its anchoring basement membrane, invade through underlying mesenchyme, and pass between endothelial cells to intravasate into the blood stream. Once in the circulation, the tumor cells must resist apoptosis driven by loss of cell contact (anoikis), escape immune recognition, and arrive intact at their ultimate destination. After they extravasate from the circulation into the target organ they must then implant, proliferate, and induce angiogenesis in order to survive and grow in a foreign and presumably "hostile" environment.[55] Each of these steps, therefore, represents a potential point of vulnerability and target for therapeutic intervention.

SEED AND SOIL OVERVIEW

Since Paget's first description of the seed and soil hypothesis regarding cancer metastasis in 1889, there has been much debate and investigation into the factors that ultimately drive metastatic deposits into their ultimate locations.[60] Put briefly, the seed and soil hypothesis maintains that cancer cells (the seed) metastasize to locations that are biochemically and physiologically favorable for implantation and growth. Given that the cause of death for most patients with cancer is the development of disseminated cancer, the understanding of metastasis is an essential prerequisite to developing novel therapeutic strategies that will significantly impact patient outcomes.

The modern day understanding of metastasis seems to support Paget's theory although our understanding of specific mechanisms driving this phenomenon is incomplete. Lung, renal, breast, melanoma, and colorectal cancers have a propensity for brain metastasis [Table 1]. However, other cancers, such as prostate, ovarian, uterine, thyroid, liver, bladder, gastric, skin, and pancreatic neoplasms tend not to metastasize to the brain.[90] To invoke Paget's theory, the circulating tumor cells (CTCs) of certain cancers (the "seeds") seem to survive in the soil of the brain for colonization and subsequent growth, relative to other cancers [Tables 1 and 3]. What about the brain provides an attractive versus hostile growth environment or "soil" for metastatic deposition and growth [Table 1]? Conversely, what is it about the seed that influences its metastatic potential in general and its specific capacity to colonize and thrive in the brain [Table 2]? Finally, why is it that some cancers like lung and melanoma, metastasize to the brain usually within 1 year of primary cancer diagnosis while others, like breast, often take more than 1 year to involve brain.[10]? The understanding of these processes in brain metastases is not academic, as patients now often die of their brain disease even in the setting of controlled systemic cancer.[10,79]

Of note, an alternative hypothesis proposed by Ewing in 1928 suggested that metastasis could be explained purely by mechanical and circulatory factors and as such, the seed and soil hypothesis was unnecessary.[22] However, seminal experiments by Fidler showed that upon direct injection of various melanoma cell lines into the internal carotid artery, some cell lines formed metastases in the brain parenchyma while others formed only in the leptomeninges despite all cell lines achieving circulatory arrest in the brain microvaculature.[23-25] Almost 120 years later, such data gives credence to Paget's hypothesis that seeds must find their appropriate soil.

Table 1: Incidence of brain metastases organized by primary tumor[16]

Primary site	Incidence of brain mets (%)
Lung and bronchus	19.9
Renal	6.5
Melanoma	6.9
Breast	5.1
Colorectal	1.8

Table 2: Common sites for metastasis.[10] This data demonstrate the importance of the soil for metastatic spread

Common metastatic sites	Common tumors
Bone	Breast
	Prostate
	Lung
	Kidney
Lung	Breast
	Bladder
	Colon
	Kidney
	Head and neck
	Melanoma
Liver	Cutaneous melanoma
	Lung
	Colorectal
	Breast
	Neuroendocrine

Table 3: Adapted from Chu[13] and Weiss.[90] In this autopsy study, Weis addressed the differences between seed and soil and blood flow theories of metastasis by investigating the incidence of metastatic lesions while correcting for blood flow to generate a metastatic efficiency index. Interestingly, prostate and breast exhibited a "friendly" interaction with brain while ovarian, prostate, stomach, and bladder exhibited a "hostile" interaction with brain"

Primary cancer site	Kidney	Brain	Bone	Skeletal muscle	Skin	Heart	Thyroid	Adrenal
Bone	-	-	-	-	-	/	-	↑
Breast	-	-	↑	-	-	-	↑	↑
Cervix	-	-	-	-	↓	/	↑	↑
Colorectal	-	-	-	↓	-	-	-	↑
Esophagus	-	-	-	-	↓	/	↑	↑
Kidney	-	-	-	↓	-	-	↑	↑
Lung	-	-	-	/	/	/	-	↑
Osteosarcoma	↓	↓	-	↓	/	↓	-	↓
Ovary	↓	↓	-	/	-	-	↑	↑
Pancreas	-	-	-	-	-	-	-	↑
Prostate	-	-	↑	-	↓	↓	-	↑
Stomach	-	↓	-	↓	-	/	-	↑
Testis	-	-	-	↓	-	/	-	↑
Thyroid	-	-	-	-	-	/	-	↑
Bladder	-	↓	-	-	-	/	↑	↑
Uterus	-	-	-	-	↓	/	↑	↑

↑: Friendly (increased incidence than would be predicted by blood flow alone), ↓: Hostile (decreased incidence than would be predicted by blood flow alone), -: Neutral, /: Not reported

CANCER STEM CELLS AS SEED

A starting point in understanding the biology of brain metastasis is the recent proposal that the "seeds" of origin for metastasis possess properties of cancer stem cells. Recent data have suggested that the metastatic seeds acquire their phenotype in a multifaceted way, with changes occurring both early and late in oncogenesis, as a result of ongoing selection pressure that promotes increased malignancy.[34,35,55,57,73] One intriguing hypothesis posits that these resulting metastatic cells are actually related to cancer stem cells if not cancer stem cells themselves.[4,55] As background, the cancer stem cell hypothesis proposes that only a very small population of tumor cells is capable of tumor initiation and self-renewal.[50] Moreover, the theory implies that elimination of these cells is a fundamental requirement to the successful treatment of cancer. These cells are not discretely identifiable but can be enriched using a variety of markers.[12,64,70,77] Of note, stem cells are capable of motility, initiation of angiogenesis, invasion, survival during circulation, and an ability to survive at distant sites by interacting with the new microenvironment. Moreover, they are able to evade growth suppressors and resist apoptosis. Finally, these cells can clonally expand resulting in cancer growth. These phenotypic attributes thus become salient when considering each of the steps in the cascade of events required for metastatic success.[13,55] Moreover, acquisition of a malignant stem cell phenotype might easily explain the resistance to elimination strategies such as chemotherapy, local antigrowth signaling and immune attack by the host.[25,54]

IMPLICATED GENES

There are a host of genes that have been implicated in metastasis, and many of them are essential to cytoskeletal maintenance and/or extracellular matrix assembly [Table 4]. For example, in melanoma, the gene RhoC (a GPTase) enhances metastasis when overexpressed, suggesting that this gene may be essential to tumor invasion.[14,67] Similarly, matrix metalloproteinases (MMPs) are critical to tumor cell intravasation from their native origin and extravasation into their ultimate site of metastasis (Table 4 describes some commonly studied genes associated with metastasis). Quick perusal of this list implicates genetic mechanisms attributed to embryologic stem cell-like phenotypes *vis a vis* cancer stem cells. Moreover, it is likely that rather than undergo individual alterations in these genes and others, changes in global gene expression profiles in premetastatic and metastatic cells are driven by complex yet stereotypic cellular paradigms. Of note, the phenotypic changes and features required for each step of a successful metastatic event are relatively stereotypic yet can map to myriad molecular genetic changes. One such implicated paradigm includes the epithelial to mesenchymal transition (EMT).

EMT

The first requirement for brain metastasis is intravasation, the escape of a cancer cell from its native tissue into the blood stream. Recently it has been recognized that the initial steps in this process whereby normally constrained

Table 4: Genes associated with increased metastatic potential

Gene	Cancer site (primary)	Role and implications
RhoC	Melanoma	Regulates remodeling of actin cytoskelton during invasion. Important for tumor cell invasion
LOX	Breast Head and neck	Increases invasiveness of hypoxic human cancer cells
VEGF	Lung Breast Melanoma Colon	Angiogenic growth factor
CSF1	Breast Lung	Stimulates macrophage growth and release of growth factors
ID1	Breast Lung	Involved in matrix remodeling
TWIST 1	Breast Gastric Rhabdomyosarcoma Melanoma Hepatocellular	Causes loss of E-cadherin mediated cell-cell adhesion, activates mesenchymal markers, and induces cell motility via EMT
MET	Renal cell	Mitogenesis, morphogenesis, motogenesis
MMP-9	Colorectal Breast Melanoma Chondrosarcoma	ECM degradation
NEDD9	Melanoma	Acquisition of metastatic potential
LEF1	Lung	Transcriptional effecter; knockdown inhibits brain metastasis
HOXB9	Lung Breast	Knockdown inhibits brain metastasis
BMP4	Lung Colorectal	Component of EMT
STAT3	Melanoma	Transcription factor whose inhibition reduces metastasis

Source: Rahmathulla *et al.*[67], EMT: Epithelial to mesenchymal transition

and immotile epithelial cells adopt invasive mesenchymal characteristics recapitulate the epithelial to mesenchymal transition (EMT known to occur during tissue morphogenesis and wound healing. A step wise series of cellular changes occur during metastatic EMT including loss of cell–cell contact, alteration of cell polarity, reorganization of the actin cytoskleleton, detachment from and invasion through the basement membrane, and migration through the mesenchyme. These changes are required for intravasation, - the cellular egress through the microvascular endothelium into the blood stream.[5,38,63,67,78,83-85,97]

Predictably, the molecular regulation of EMT is achieved through a complex regulatory network driven by key "master regulators". One implicated gene is TWIST1, a transcription factor important in embryonic development also expressed in many cancers ranging from prostate, bladder, and even gliomas.[11,21,88,92] In part its mechanism of action is to down regulate E-cadherin mediated cell adhesion thereby promoting a motile and mesenchymal phenotype. The loss of E-cadherin is associated with upregulation of N-cadherin and this so-called "cadherin switch" is considered a canonical indicator of metastatic EMT. Other traits conferred by EMT include the ability to loosen adherens junctions, express matrix degrading enzymes, resist apoptosis, and undergo morphological conversion.[67] Interestingly, hypoxia, which affects malignant neoplasms as they outstrip their vascular supply, is known to activate EMT signaling cascades.[5,36] Phenomenonlogically, it is quite possible that as some seeds undergo EMT as a response to hypoxia and metastasize, their subsequent recapitulation of the primary tumor derives from reestablishment of normoxic conditions and reversal of EMT via MET (see below). Other molecules important for EMT include TGF-beta, HGF, EGF, IGF, FGF, the Wnt, NOTCH, PI3k/AKT, and Hedgehog pathways, as well as other "master" transcription factors like Snail/Slug and SMADS.[67,81,86] Of importance, the mechanisms that activate EMT also promote the acquisition of stem-like properties for the purposes of metastasis thereby providing a mechanism for acquisition of the malignant stem cell phenotype.[87]

The importance of various growth factors in EMT also indicates the importance of an extensive cross-talk between cancer cells and the surrounding stroma.[53] Some of these interactions may be facilitated by stromal cells such as cancer associated fibroblasts, pericytes, or even astrocytes.[19,58] For example, astrocytes help produce

a rich environment of cytokines and growth factors such as SDF-1 alpha, IL-1, IL-6, IFN-gamma, TNF-alpha, PDGF, and TGF-beta.[13,96] Of note TGF-beta is a critical growth factor implicated in EMT. Moreover, PDGF and TGF-beta synergize in aiding cancer cell growth, invasion, and metastasis.[56] Therefore it is quite possible that just as EMT is important in generating the initial metastatic phenotype, the elaboration of brain-derived pro-EMT factors might also regulate an EMT phenotype in cancer cells after successful implantation within the brain. The significance of a potential "secondary" phase of EMT in the brain is unknown but could have unrecognized importance for establishing a metastatic niche essential for growth or regulating the invasive character and treatment responsiveness of individual metastatic deposits.

CIRCULATING TUMOR CELLS

Once the seeds of the primary cancer disseminate via extravasation into the bloodstream, mediated perhaps by EMT, they must then survive in the circulation and eventually deposit in their ultimate locations. CTCs have been demonstrated in many types of solid tumors[5,61,62] and quantification of CTCs in breast, colorectal, and pancreatic cancer has been shown to correlate with survival.[6,15,17] An intriguing area of active research is whether the identification and molecular characterization of these cells before clinical manifestation could better predict metastatic risk and facilitate individualized therapeutic strategies to inhibit target organ colonization and metastatic growth.

Of note, the activation of an EMT is favorable for the CTC population. The ability to survive in the circulation by avoiding aniokis derives from mesenchymal attributes similar to those present in circulating hematopoietic cells. Interestingly, it has been reported that the presence of mesenchymal markers on CTCs more accurately predicted worse prognosis than the expression of cytokeratins (another markers of CTCs).[5,28,44,68] Moreover, patients with CTCs in the blood and reduced E-cadherin expression in the primary tumor were also observed to have the shortest disease-free survival.[52,75] Finally, downregulation of E-cadherin and upregulation of mesenchymal markers such as N-cadherin and vimetin have been shown in prostate, breast, and lung cancers thereby providing reasonable evidence of an EMT-like process driving, or at least persisting, in the migratory stage of cancerous seed cells.[2,33,43]

MET

While EMT is critical for early events in metastasis, most metastatic lesions are morphologically indistinguishable from parent tumors and lack evidence of a persisting EMT phenotype. This suggests that metastatic cancer cells at some point revert from the mesenchymal form required for intravasation back to their native epithelial phenotype. This process, postulated to represent a phenotypic reversion of EMT, has been termed mesenchymal to epithelial transition (MET).[3,9,29,91,94] In support of MET are observations that metastases phenotypically resemble the corresponding primary tumors, and that in the majority of cases they express epithelial markers.[5] To achieve this transformation, the soil of the ultimate metastatic destination must not only be hospitable to the CTC itself, but it must also be able to provide the necessary stromal support for the CTC to undergo MET.[56] Molecularly, this corresponds with the reexpression of E-cadherin and morphologic change to a more epithelial phenotype.[91] As such, the propensity of certain tumors for metastatic spread to the brain may be partly explained by the possible support these CTCs receive not only for implantation but also for the purposes of undergoing MET. Growth factors implicated in driving MET include FGF, EGF, BMP, as well as the Wnt and Akt pathways,[94] which may be modulated by astrocytes in supporting stromal niches. In contrast, the pro EMT signaling TGF-beta, expressed by brain astrocytes, prevents MET; as such, colonization of circulating tumor stem cells in brain through a MET-like process requires heretofore unidentified stromal support but may be critical in the process of tumor propagation after implantation.[56] Alternatively, CTCs may be in an EMT/MET equilibrium in the brain where factors like regional hypoxia, stromal components, and nearby supporting cells play a role in keeping some cells in a stem-like state capable of self-renewal through an EMT-like program while other cells regain epithelial characteristics to reestablish the primary tumor through a MET-like program as the soil allows [Figure 1]. If present, the dynamics of an EMT/MET equilibrium might be of clinical relevance in the context of invasive growth patterns of individual lesions and variable growth and treatment responses seen across different types of brain metastasis even within individual patients.

SOIL

Upon arrival to a distant site, the metastatic cell needs to establish a foothold in its new microenvironment. Interestingly, this engagement is not easy; the efficiency of forming metastatic deposits is poor once CTCs are present in the blood stream. For example, though tumor cells in the bloodstream are a common finding in metastatic disease, less than 0.01% of CTCs succeed in forming metastases.[23,24,54] Moreover, direct injection of millions of tumor cells into the vena cava via portosystemic shunts in patients with ascites from ovarian cancer resulted in very few eventual secondary cancers.[82] Therefore, the survival of a CTC in a new environment

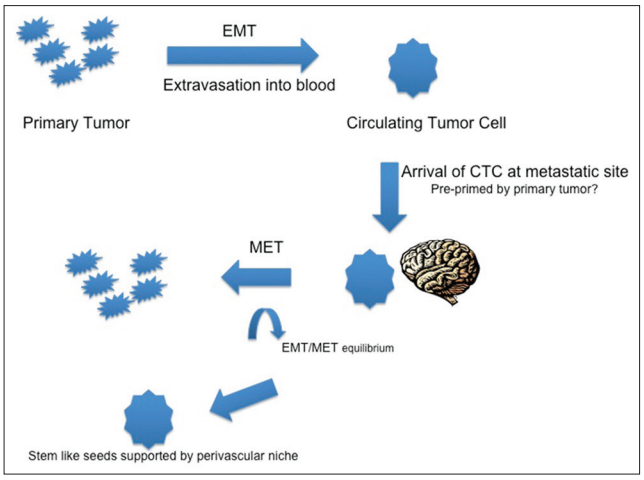

Figure 1: Schema for EMT/MET pathobiology of metastasis. Cells (seeds) at primary tumor site undergo EMT-like program to acquire metastatic potential. Once circulating they must find a hospitable microenvironment to implant. There, they may exist in an EMT/MET equilibrium that allow maintenance of cancer stem cell-like populations for cancer renewal and reestablishment of primary tumor phenotype via an MET-like program. All of these steps are of potential treatment significance. Notably, current cancer chemotherapeutic regiments generically target the growth of cancer cells and do not target the various phases of metastasis as depicted. Moreover, current treatment paradigms including radiation do not necessarily target cancer stem cells either, as they may reside in protective niches within their metastatic sites (soil)

appears to require specific trophic interactions with its surroundings. This phenomenon was demonstrated in a study where despite widespread circulatory distribution of mouse melanoma cells achieved after IV injection into syngeneic hosts, metastasis only formed in the lungs or ovaries.[31]

Therefore, success of the seed requires successful interaction with its soil. Such interactions may begin with proteins such as ezrin, which helps link the plasma membrane to its actin cytoskeleton and thus facilitate cell surface adhesion.[42,55,95] Likewise, cell–cell interactions facilitated by integrins, ICAMS and cell–stromal cell (fibroblasts, macrophages, endothelial cells) interactions facilitated by paracrine pathways help upregulate angiogenesis and prosurvival pathways necessary for successful metastatic spread. More recently, it has been shown that metastases can bring their own soil (which can be detected in metastatic resection specimens) in the form of lung carcinoma associated fibroblasts, whose presence as a cotravelling partner can greatly increase the efficiency of metastasis.[19] Interestingly, the soil is not just facilitative but augmentative given data that metastatic cells have substantially increased tumorigenicity when grown *in vivo* compared with *in vitro*.[30] Again, important growth factors produced in the stroma include TGF-beta, EGF, FGF, Wnt pathways, CXL12, and IL-6.[67] Invasion of cancers can be further enhanced by stroma macrophages, which stimulated by CSF-1 secreted by tumor cells, may supply matrix degrading enzymes such as MMPs and cystein cathepsin protease.[66,67] Specific genes implicated in the seed–soil interaction depend on the primary tumor. For example, in breast, CXCR4 overexpression has been noted in metastazing tumor cells. Interestingly, CXCL12 is a chemotactic protein preferentially expressed in the stroma of typical target organs of breast mets, such as brain, bone, etc., thereby lending credence to the seed–soil hypothesis as well as suggesting a potential avenue of therapy.[39]

New evidence now suggests that there may also be a priming of the metastatic microenvironment in advance of the actual deposition of metastatic tumor cells.[40,65] In this hypothesis, tumor cells secrete factors that drive hematopoietic progenitor cells to sites of future metastatic spread. These cells then can "prepare" the microenvironment to facilitate metastatic deposition. Separately, the genetic background of the individual may be critical to metastatic success, with individual polymorphisms of the patient either facilitating or suppressing metastatic success.[34] Taken together, these observations suggest that the risk for metastatic progression is in part defined by the genetics of the patient, genetic changes that develop early in the process of tumor development, and the subsequent and incremental emergence of cells within the tumor that possess the cellular armamentarium needed for metastasis.

BRAIN AS SOIL

The brain itself provides unique challenges to the metastatic cell. Difficulties in colonizing the brain are a consequence of its lack of lymphatic drainage and the presence of its robust blood–brain barrier, which even restricts serum proteins unless shuttled by active transport.[54] Metastatic cells may get around this problem by disrupting the endothelium to gain entry. Once there, the endothelium may be further compromised as the tumor grows, as the necrosis associated with growing tumors may encourage leakiness of the blood brain barrier (possibly mediated by VEGF) via opening of tight junctions and damage to the endothelium itself.[25] Moreover the vasculature of the brain itself has recently been implicated as the essential "soil" for metastatic success. In a recent study,[8] the vast majority of micro-metastases demonstrated vascular cooption with little evidence for any neurotropic growth. Additionally, this interaction was adhesive in nature implicating the vascular basement membrane as the active substrate for tumor cell growth. Finally, blockade of the B1 integrin subunit prevented adhesion to the basement membrane and attenuated metastasis.[8] Two more recent studies have indicated that these same endothelial cells actively help metastatic cells transmigrate into the parenchyma.[45,51] In total, this data indicates that the cerebral vasculature may provide the home base from

which further extravasation into the parenchyma occurs. Interestingly, this "perivascular niche" is also common to brain tumor stem cells and glioma stem cells, thus providing further evidence that metastatic seeds may develop from stem-like populations.[7,8,76]

Other research has demonstrated how metastases to the brain hijack the normal brain parenchyma rather than rebuild their native tissue microenvironment. For example, recent data implicates astrocytes as critical protectors of the tumor cell. In an elegant experiment, Lin *et al.* showed that through physical contact via their podia, astrocytes can protect tumor cells from 5-fluorouracil and cisplatinum-induced apoptosis.[49] Furthermore, these astrocytes produce a diverse cocktail of cytokines, like SDF-1 alpha, IL-1, IL-3, IL-6, IF-gamma, TNF-alpha, TGF-beta, and PDGF-1, all of which may be essential in helping establish the metastatic niche.[13] Separately, the tumor's management of vascular endothelial growth factor may be critical in its ability to coopt existing blood vessels in the brain. In one study, tumor cells with high rates of metastatic spread to the brain expressed higher levels of VEGF activity than tumor cells with low rates of brain metastasis.[93] Moreover, transfecting human lung and colon cancer metastases with antisense VEGF165 significantly decreased the rates of brain metastasis and blood vessel ectasia. In contrast, transfection of human squamous lung cancer with sense-VEGF did not enhance metastatic formation.[25,93] Interestingly, in mouse studies with melanoma, lung, and colon carcinomas metastatic to brain, angiogenesis did not occur by growth of new vasculature but by splitting of preexisting dilated blood vessels.[25,26] This data are in contradistinction to metastasis in other parts of the body, where the mean vessel density around the periphery of the tumor correlates with the aggressiveness of the disease.[89] In total, this data has shown that VEGF is necessary but not sufficient for the production of brain metastases. This may also explain some success of treating metastatic disease with monoclonal antibodies against VEGF, such as bevacizumab.

THERAPEUTIC IMPLICATIONS

Among the reasons for treatment failure in cancer therapy, some of the most compelling include the inability of current therapies to target the seed cells or cancer initiating cells that renew a neoplastic population after the sensitive population has been treated with either surgery or chemoradiation.[1,69] One of the reasons for this inability to target the cancer stem cells may be that they reside in protected niches within the brain. This concept has been shown to be true in gliomas, as Calabrese *et al.* demonstrated that growth of glioma cells is severely diminished by targeting the "vascular niche" in which endothelial cells protect glioma stem cells by secreting stem cell survival factors.[7] Alternatively, one therapeutic strategy would induce the cancer stem cells to differentiate, subsequently lose their stem-like properties, and perhaps become more treatment sensitive as a result.[27,46] Likewise, targeting the tumor microenvironment (soil) may be a way to effectively treat metastatic spread. For example, macrophage knockout mice exhibit a reduced rate of tumor growth and a significant decrease in metastasis compared with controls while overexpression of macrophage stimulating factors accelerates tumor growth.[47,48] Additionally, therapy aimed at affecting tumor cell–stroma interaction by disrupting molecular cross talk (VEGF, FGF, PDGF) may be effective.[37,71]

UNANSWERED QUESTIONS

A peculiarity with the seed and soil hypothesis involves the strikingly different behaviors of certain cancers and their seeds with the same soil. For example, breast adenocarcinoma and small cell carcinoma of the lung are well-known to metastasize to brain. However, breast metastases may be detected years after remission of primary disease while lung metastases typically appear in close proximity to the initial diagnosis of the primary lesion.[32,41] If both seeds acquire malignant phenotypes required for metastatic spread, what then underlies the drastically different behaviors in the two tumors? Might there be something about the soil in the brain that can induce a dormant state in breast metastases but not lung? If so, what in the soil induces these cells out of dormancy? Alternatively, is dormancy something specific to the seed cell itself?

Additionally, are cancer stem cells really just manifestations of an active EMT program? Do these seeds ultimately revert to their primary tumor phenotype via MET or are these cells in a state of perpetual EMT/MET equilibrium where the balance is determined by externalities such as the soil? Relatedly, will therapies that target this embryologic program prove fruitful in cancer therapy?

Finally, as discussed earlier, the blood–brain barrier is not intact, particularly in large tumors, though the degree of leakiness is variable. As such, why are chemotherapies ineffective if drug can cross into the tumor? Is it because of inadequate drug levels or because of protective perivascular niches that isolate cancer stem cells from potentially toxic substances?

CONCLUSIONS

As previously recognized by others in the modern era,[23-26] the present update further demonstrates the contemporary relevance of Paget's seed and soil hypothesis. Noteworthy advances are the recognition

of cancer stem-like cells as "seeds" for brain metastasis and the pleiotropic effects of an EMT-like program to promote the inciting events of the metastatic cascade as well as the generation and/or maintenance of stem-like phenotypes also evident in CTCs. Additionally, MET is posited to derive from metastatic cell interactions with the "soil" or metastatic niche in the brain to promote survival and growth coincident with reversion from mesenchymal to the parental cancer phenotypes. Built on the foundation of the seed and soil hypothesis, these new insights into the basic cellular and molecular mechanisms driving brain metastasis are anticipated to improve patient outcomes and quality of life by development of techniques to diagnose, prognosticate and treat the ever increasing number of cancer patients suffering from metastatic disease to the brain.

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