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Revisiting the seed and soil in cancer metastasis

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Abstract

Metastasis remains the overwhelming cause of death for cancer patients. During metastasis, cancer cells will leave the primary tumor, intravasate into the bloodstream, arrest at a distant organ, and eventually develop into gross lesions at the secondary sites. This intricate process is influenced by innumerable factors and complex cellular interactions described in 1889 by Stephen Paget as the seed and soil hypothesis. In this review, we revisit this seed and soil hypothesis with an emerging understanding of the cancer cell (i.e. seed) and its microenvironment (i.e. soil). We will provide background to suggest that a critical outcome of the seed–soil interaction is resistance of the stresses that would otherwise impede metastasis.

Keywords

Metastasis; Microenvironment; Stress; Seed; Soil

1. Introduction

1.1. Problem of metastasis

The cause of death for the vast majority of cancer patients is the development of metastatic lesions at sites distant from that of the primary tumor. Metastasis describes both the process of cancer spread (i.e. the verb, describing the events that characterize the spread of cancer) and the resultant secondary cancer (i.e. noun, describing the actual metastatic lesion). Since most cancer patients present with localized disease that is effectively managed with multimodality therapies including surgery, radiation, and chemotherapy, the development of metastasis at distant secondary organs must involve the dissemination of metastatic cells before patients present with a primary tumor. Based on the work of several groups, it is believed that the process of metastasis (i.e. the verb) involves tumor cells leaving the primary tumor through a well-regulated lysis of surrounding stroma. These cells must pass through the tumor basement membrane and then through or between endothelial cells in order to enter the circulation. While in the circulation, a tumor cells must resist the process of anoikis (programmed cell death associated with loss of cellular contact), evade immune recognition, cope with the sheer physical stress of the circulatory system, and eventually arrest at a distant organ. At the distant site, the cell must exit the circulation, survive the

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stresses of a new and likely hostile microenvironment, proliferate, induce angiogenesis and/or co-opt existing blood vessels, and then successfully grow into a measurable metastatic lesion (Steeg and Theodorescu, 2008).

1.2. Clinical features/description

The timing, pattern and sites for the spread of cancer are in part defined by the specific cancer type. The route of spread of cancer may include blood stream (hematogenous), lymphatic vessels, or third space extension (i.e. ascitic fluid dissemination as seen in ovarian cancer). The site of distant metastasis may include regional lymph nodes, or visceral organs such as lungs, liver, brain, and bone. While Weiss et al. (1988) have shown that the primary site of metastases tends to occur at the first capillary bed encountered, it is increasingly believed that the specific site of distant metastasis is not simply to be due to anatomic location of the primary tumor or proximity to secondary sites but rather, involves interactions between tumor cells and the local microenvironment at the secondary site.

For many reasons, metastatic lesions are often not amenable to the surgical cures achieved in the management of the primary tumor. First, the development of metastases at distant secondary organs is often so widespread that surgery is not possible. In addition, the organs in which metastases develop may not be able to accommodate the necessary wide surgical margins needed for cure (i.e. brain). For many cancers, metastatic lesions themselves demonstrate increased resistance to conventional treatment modalities (i.e. chemotherapy). This resistance to therapy may be acquired as a result of past treatment of the patient or may be an innate feature of cells that have successfully negotiated the metastatic process. Based on the challenges that metastatic disease presents, new treatment options are needed in order to decrease morbidity and mortality.

1.3. Revisiting the seed and the soil

Stephen Paget’s seed and soil hypothesis suggested that the sites where metastases occur are defined not only by the tumor cell (“seed”) but also the microenvironment of the secondary metastatic site (“soil”). Recent data have shed new light on the acquisition of the “seeds” of metastasis, suggesting that some of the features of this tumor cell phenotype are conveyed early in the process of oncogenesis, whereas others are selected for during cancer progression (Scheel et al., 2007; Talmadge, 2007). Indeed, many of the features of the tumor cells (“seed”) now appear to be shared with primitive stem-like cells capable of not only tumor-initiation but also metastasis (Mehlen and Puisieux, 2006; Croker and Allan, 2008; Vermeulen et al., 2008). An attractive link between the tumor-initiating cell (cancer stem cell) hypothesis and metastasis is the programming and ability of these stem-like, self-renewing and multipotent cells to resist stress, perhaps facilitated by their abilities to engage and develop connections with specific microenvironments (“soil”). In this review we will consider new data that describe the tumor cell, its microenvironment, and connections between the two as a critical means to resist the stresses that cells encounter during metastatic progression.

2. Pathogenesis

2.1. Characteristics and emergence of the tumor cell (the “seed”)

The emergence of the metastatic phenotype within a primary tumor has been explained as a process that happens late in carcinogenesis. This hypothesis holds that tumor cells possessing the metastatic phenotype represent a very small fraction of cells within a heterogeneous primary tumor and that over time, a small proportion of tumor cells gain the attributes necessary for metastasis (Fidler and Kripke, 1977). In testing of this hypothesis, Varmus and colleagues were able to show untransformed mammary cells that had been delivered to mice by tail-vein injection and were able to accomplish many of the steps required for metastasis. Induction of MYC and mutant Kras oncogenes in these cells after their arrival and establishment in the lung yielded tumor after three to four weeks (Podsypanina et al., 2008). Additionally, another possibility has suggested that the emergence of the metastatic phenotype may not only occur as a late event in metastatic progression but rather may be linked to early oncogenic events that also drive primary tumor formation (Scheel et al., 2007). To further complicate this analysis, work by Hunter et al. (2003) has further suggested that the propensity for a primary tumor to metastasize may even precede primary tumor formation and is in fact related to the patient’s germline genetics. In a sense, these data return us to Paget’s hypothesis by suggesting that specific genetic determinants of the host (i.e. soil) contribute to the success of the metastatic process (i.e. seed). Taken together the work by several groups suggests 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.

2.2. Cancer stem cell/tumor-initiating cell hypothesis

A re-emerging hypothesis in the field of cancer biology is that cancers emerge from cells with primitive or stem-like features (see recent review (Lobo et al., 2007)). The basis of this hypothesis is that only a very small population of tumor cells is capable of tumor-initiation and self-renewal (stem-like). Although these tumor cells are not discretely identifiable, the population can be enriched using markers commonly found on primitive or stem-like cells (Singh et al., 2004b; Prince et al., 2007; Ricci-Vitiani et al., 2007; Cho et al., 2008). The recent finding of stem cell markers in many adult solid tumors has refueled an interest in this hypothesis that extends beyond pediatric and blood borne cancers where this model was first suggested. Interestingly, cells with stem-like properties include many of the features of the metastatic cell (“seed”). For example, embryonic and adult stem cells are capable of motility, invasion, survival during circulation, extravasation at secondary sites, dormancy and perhaps most importantly an ability to engage and interact with appropriate cellular and non-cellular partners at a secondary location to ensure their continued survival and eventual proliferation (seed–soil) (Ozturk et al., 2004). Since primary tumor-initiating cells may exhibit many of the features of the metastatic phenotype, these cells may be amenable to ‘reprogramming’ through secondary genetic or epigenetic events (acquired or selected). The result of this reprogramming may be selection of an optimal metastatic phenotype. These cells are thought to be crucial to the initiation and dissemination of several cancer types and,

with their ability to resist conventional therapies, are likely to play a significant role in the metastatic process (Barnhart and Simon, 2007).

2.3. Stress and metastatic success

Of the determinants of success in metastasis, the tumor cell's ability to resist the stresses associated with the multiple steps in the metastatic cascade and survive at distant sites may be paramount (Hedley et al., 2008). Indeed, this resistance of stress may be a feature common to stem-like cells, tumor-initiating cells, and metastatic cells. Stresses such as hypoxia, reactive species (RS), inflammation, nutrient deprivation, and pH can be a challenge to the growth and progression of a cancer and are usually and initially detrimental to the tumor's survival. However, such stresses may also provide a selective pressure favoring growth of more metastatically 'fit' cells (Witz and Levy-Nissenbaum, 2006). These stresses are likely to be felt within the primary tumor, during the process of metastasis, and at the distant secondary metastatic sites (Fig. 1). The following section will summarize some of the stresses listed above that are faced by metastatic cells and the mechanisms that allow for accommodation to these stresses by successful metastatic cells.

2.3.1. Hypoxia—Low oxygen tension, or hypoxia, frequently occurs during tumor progression and metastasis. Tumor cells, unlike normal cells, are able to survive adverse hypoxic conditions through a diversity of mechanisms (Rong and Vande Woude, 1994; Reynolds et al., 1996; Maulik and Das, 2002; Birchmeier et al., 2003; Semenza, 2003; Xie and Huang, 2003). The transitions between "oxic" states can be additionally challenging for cells to endure. Cells that are able to resist the negative effects of low oxygen conditions and then competitively flourish in hypoxic environments have been shown to be highly dependent on the expression of the transcription factor hypoxia-inducible-factor-1 (HIF-1) (Semenza and Wang, 1992). Indeed, several studies have shown an association between hypoxia, tumor progression, stem-ness and metastasis (Young et al., 1988; Brizel et al., 1996; Hockel et al., 1996; Jang and Hill, 1997; De Jaeger et al., 1998; Rofstad and Danielsen, 1999; Rofstad et al., 2000; De Jaeger et al., 2001). Cancer cell adaptation of the HIF-1 pathway may occur secondary to genetic mutations or epigenetic events (Kapitsinou and Haase, 2008). HIF-1 promotes the expression of several metastasis-associated genes by binding to hypoxia-responsive elements within their respective promoters. Such HIF-1 responsive genes include NOS (McQuillan et al., 1994; Melillo et al., 1995), IGF-II (Kim et al., 1998), CXCR4 (Semenza, 2003), and VEGF (Shweiki et al., 1992; Minchenko et al., 1994). Increased expression of some of these genes (VEGF and CXCR4, for example) can also help to counteract hypoxia by increasing oxygen levels creating a more suitable environment for tumor growth (Xie and Huang, 2003). HIF target genes are also important for stem cell growth and maintenance and have recently been shown to play important roles in cancer (Keith and Simon, 2007). Hypoxia may also be crucial for the creation and/or maintenance of the undifferentiated state of cancer stem cells (Keith and Simon, 2007). Recently, Gustafsson et al. (2005) demonstrated that hypoxia promoted an undifferentiated cell state through Notch signaling. Other genes including hTERT, the catalytic component of human telomerase (Nishi et al., 2004), the multi-drug resistance ABC transporter Bcrp/ABCG2 (Krishnamurthy et al., 2004), and the MET tyrosine kinase receptor (Boccaccio and

Comoglio, 2006), are HIF regulated, are expressed in both cancer cells and stem cells, and contribute to the metastatic phenotype of cells.

2.3.2. Reactive species—The transient and vacillating hypoxia of the tumor microenvironment confounds the problem of hypoxia during re-oxygenation through the formation of reactive oxygen or nitrogen species (Xie and Huang, 2003). These reactive species may have contrasting effects on metastasis (Ambs et al., 1997; Xie and Fidler, 1998; Xie and Huang, 2003). In some cancers the formation of RS, i.e. nitric oxide (NO), contributes to the metastatic phenotype while in other cancers, reduced RS are linked with metastasis (Yamamoto et al., 1994; Pipili-Synetos et al., 1995; Iwasaki et al., 1997; Yamamoto et al., 1998). Mechanistically, NO expression is associated with altered expression of many mediators of metastasis, including VEGF-C (Radomski et al., 1991; Franchi et al., 2006; Nakamura et al., 2006; Brideau et al., 2007), MMP9 (Marcet-Palacios et al., 2003), p53 (Cook et al., 2004), and HIF-1 α (Kimura et al., 2000; Sandau et al., 2001). Intratumoral RS may also contribute to genomic instability associated with advanced cancers and may play a trophic role in tumor progression (Ambs et al., 1997; Xie and Fidler, 1998; Radisky et al., 2005; Halliwell, 2007). The observation of reduced RS in metastatic versus primary tumors may represent an adaptive advantage of some metastatic cancers to effectively manage the detoxification of RS (Xie and Huang, 2003).

2.3.3. Inflammation—The link between inflammation and cancer has been recognized for many years now. For example, in, 1986, Dvorak described cancer as “wounds that fail to heal” (Dvorak, 1986). Both inflammatory mediators and cells involved in the inflammatory response react against cancer cells and contribute to tumor progression. As such, macrophages represent an important mediator of the cancer inflammatory environment and have been show to increase the incidence of metastases in both in vitro and in vivo (Gorelik et al., 1982, 1985). Their influence is determined, in large part, by the local cytokine profile. As part of the innate immune system, macrophages can be potent anticancer cells in a GM-CSF rich environment (Gillessen et al., 2003). Conversely, successful cancers appear to create a cytokine environment that is dominated by CSF-1 rather than GM-CSF (Nowicki et al., 1996). In the CSF-1 dominated environment, macrophages contribute to the metastatic phenotype through many paths, including generation of RS, initiation of invasion and angiogenesis (Coussens et al., 2000; Huang et al., 2002; Pollard, 2004; Chen et al., 2005), and contribution to genomic instability (Condeelis and Pollard, 2006). In this way, successful metastatic cells appear to engage their microenvironment and recruit beneficial rather than deleterious populations of inflammatory cells to create a more “cancer hospitable environment.” Beyond the macrophage, other inflammatory cells contribute to the metastatic phenotype of cancers, including lymphocytes, neutrophils (Aeed et al., 1988; Welch et al., 1989; Caruso et al., 2002), mast cells (Brideau et al., 2007), T-regulatory cells (Khazaie and von Boehmer, 2006; Wahl et al., 2006; Teicher, 2007), and platelets (Karpatkin et al., 1988; Borsig, 2008). These cells can negatively regulate the host immune response against cancer. This may be accomplished through production of inflammation-based products such as chemokines, ROS/RNS, cytokines, and growth factors (Reichert et al., 2002; Pawelec, 2004; Yang and Carbone, 2004). TGF-beta, which can be released by macrophages, lymphocytes, and/or platelets inhibits T-cell proliferation through suppression of interleukin (IL)-2

production (Becknell and Caligiuri, 2005; Ma et al., 2006) and blunts natural killer (NK) cell activity by interfering with IFN-gamma production (Rook et al., 1986; Bellone et al., 1995). Matrix metalloproteinases (MMPs) are a family of proteins capable of degrading the extracellular matrix. Higher levels of MMPs are often associated with enhanced tumor invasion and metastasis and can often be secreted by several of the stromal cells present in the tumor microenvironment including macrophages and fibroblasts (Stetler-Stevenson et al., 1993; Sternlicht et al., 1999; Boire et al., 2005; Deryugina and Quigley, 2006). The ability of cancer cells to evade parts of the immune response and direct inflammatory cells towards benefit involves a complex set of relationships between the tumor cells and their environment at primary and secondary sites and appears crucial to the initiation and progression of cancer.

2.3.4. Nutritional and pH stress—Deprivation of cellular nutrition and alterations in pH balance are common stresses linked to cancer. The mammalian target of rapamycin (mTOR) is a critical cell signalling mediator involved with sensing the nutritional environment of cancer cells. The mechanisms of sensing and responding to these stresses occur through signaling intermediates such as AKT and mitogen-activated protein kinase. The current dogma suggests that mTOR coordinates signals from the nutritional and stress status of a cell resulting in upregulation and activation of specific proteins to maintain cell homeostasis. For example, tumor cells are highly dependent on the targets of mTOR-mediated translation such as c-myc, VEGFR, hypoxia-inducible factor, and transforming growth factor-beta.

Reduction in the pH is often seen in tumors, and often occurs in conjunction with regions of hypoxia. One of the ways a tumor cell can manage this stress is through increased glucose metabolism, which is frequently observed in cancers. In the 1920s, Warburg (1956) reported the observation of increased aerobic glycolysis in tumor cells. This shift in cellular metabolism has recently been shown to be dependent on the M2 splice isoform of pyruvate kinase (Christofk et al., 2008). Other groups have hypothesized that the Warburg effect is a mechanism for pre-malignant cells to adapt to intermittent hypoxia (Gatenby and Gillies, 2004). GLUT proteins, which function in transporting glucose across the plasma membrane, may also play an important role in the increased glucose metabolism phenotype of tumor cells. Studies have shown that hypoxia-responsive GLUT family members GLUT1 and GLUT3 are frequently overexpressed in tumors (Binder et al., 1997; Younes et al., 1997; Smith, 1999; Medina and Owen, 2002) while GLUT12 is found distinctively in prostate and breast cancer (Chandler et al., 2003; Rogers et al., 2003).

Another family of proteins that may play a role in helping cells cope with similar microenvironmental stresses are the chloride intracellular channels (CLICs). CLIC proteins are intimately involved in maintaining electrogenic gradients across both plasma and organelle membranes and thereby also serve in regulating organelle volume and pH (Jentsch et al., 2002). Beyond managing pH, cytoplasmic CLIC4 is also a direct response gene for c-myc and p53 with consensus binding sites for each in its promoter (Fernandez-Salas et al., 1999, 2002). CLIC4 is required for p53-induced apoptosis (Fernandez-Salas et al., 2002) and knockdown of CLIC4 in tumor cell lines results in inhibited tumor growth (Suh et al., 2005). In response to various forms of stress, CLIC4 will translocate to the nucleus resulting

in cell cycle arrest and accelerated apoptosis (Suh et al., 2004). In addition, with advancing malignant progression, CLIC4 expression decreases in tumors while increasing in stromal cells (Suh et al., 2007) such as myofibroblasts (Seemayer et al., 1979; Ronnov-Jessen et al., 1995; Martin et al., 1996; Bhowmick et al., 2004). This linked expression of CLIC4 between the tumor and stromal cells is suggestive of a molecular cross-talk between the tumor and its microenvironment. The mechanisms regulating activation and intracellular transit of CLIC proteins are not well understood, but may be connected to the metastatic phenotype of cancers.

2.3.5. Heat shock proteins—An overarching mechanism by which cancer cells resist stress is through the stabilization and protection of many of the proteins described above. Heat shock proteins (Hsp) including Hsp70 and Hsp90 are molecular chaperones of many of these proteins (referred to as “client” proteins) that in many cases are linked to oncogenic and metastatic cancer phenotypes. For the most part, Hsp–client protein interactions protect these proteins from degradation, and physiologic protection of specific proteins has emerged as a physiologic means to overcome short-term cellular stressors. Several Hsp clients include many of the same proteins that are often mutated or have deregulated expression in cancer. The fusion product of the Bcr and Abl genes, $p210^{Bcr\text{-}Abl}$, is intimately involved in both acute lymphocytic leukemia and chronic myelogenous leukemia and its stability is dependent on its association with Hsp90 (Blagosklonny et al., 2001). Hsp client HER-2 is commonly overexpressed in several cancers and it too is reliant on its association with Hsp90 for stability (Xu et al., 2001). Inhibition of HSP90 can also reduce hypoxia-induced HIF1 α transcription (Hur et al., 2002). Other oncogenic proteins known to be stabilized by Hsps include mutated p53 (Zhang and Burrows, 2004) and the stress-responsive kinase Akt, a key player in cell survival and metastatic spread (Toker and Yoeli-Lerner, 2006). Overexpression of other HSPs is associated with various malignancies and/or poor prognosis in patients. For example, Tomasovic et al. (1984) was able to show that metastatic clones had a higher levels of thermal resistance and also displayed enhanced rates of synthesis of fours HSPs when compared to clones from the primary tumor. Hsp27 is correlated with poor survival in liver cancer (King et al., 2000), gastric carcinoma (Cardones et al., 2003), osteosarcoma (Uozaki et al., 2000), and colorectal cancer (Zhao et al., 2007). Enhanced Hsp70 expression is correlated with outcome in lymph node metastases in squamous cell carcinoma (Kawanishi et al., 1999) and prognosis in bladder (Syrigos et al., 2003) and breast cancer (Thanner et al., 2003). The stress-mediated induction of the ER-chaperone HSP70 family member GPR78 by the unfolded protein response can lead to tumor proliferation (Luo et al., 2006; Dong et al., 2008), chemotherapeutic resistance (Pyrko et al., 2007), survival (Ranganathan et al., 2006; Fu et al., 2007), and metastasis (Fu and Lee, 2006; Zhang et al., 2006). These data additionally link the success of metastatic cells with the ability to effectively and selectively manage the stresses of protein accumulation.

2.4. Characteristics and emergence of the tumor microenvironment (the “soil”)

Upon arrival at a distant site, the new microenvironment (the “soil”) encountered by the metastatic cell is considered to be foreign and/or inhospitable. Survival of metastatic cells at the secondary site is likely a consequence of intrinsic features of the metastatic cell and an ability to effectively engage in a molecular cross-talk with its surroundings and modulate the

environment of the secondary site. This engagement can occur by several mechanisms. One such mechanism may be through the use of the cytoskeletal protein ezrin. Ezrin is necessary and/or sufficient for metastasis in several cancers (Khanna et al., 2004; Yu et al., 2004) and links the plasma membrane as well as plasma membrane associated proteins with the actin cytoskeleton. Through this linker function, ezrin is thought to help cells resist stress (unpublished data) and improve metastatic efficiency by better enabling the cell to physically engage its microenvironment and transduce microenvironmental cues in order to respond to stress. Recent studies have suggested that ezrin contributes to metastatic efficiency through inhibition of apoptotic death experienced by most cancer cells upon their arrival at secondary sites (personal communication, C. Khanna).

Similar to ezrin's role, cell–cell interactions are likely necessary for effective modulation of secondary sites. Integrins, key mediators of cell–cell interactions, often have deregulated expression in tumor cells (Juliano and Varner, 1993; Kurschat and Mauch, 2000). This aberrant expression is thought to provide enhanced proliferative and survival capabilities (Aplin et al., 1999; Hynes, 2002; Nikolopoulos et al., 2004; Naylor et al., 2005; Reddig and Juliano, 2003) in the tumor cell's new microenvironment. Growth factor receptor–ligand interactions between tumor cells and host stromal cells can often result in an interactive signaling loop between tumor and host cells (Condeelis and Pollard, 2006; Yamaguchi et al., 2006) resulting in induction of angiogenesis (Pollard, 2004) or upregulation of pro-survival pathways (Derynck et al., 2001; Kalluri and Neilson, 2003; Bhowmick et al., 2004; Mueller and Fusenig, 2004; Kalluri and Zeisberg, 2006). Stromal cells such as macrophages and fibroblasts are often involved in such signaling loops (Forsberg et al., 1993; Condeelis and Pollard, 2006; Kalluri and Zeisberg, 2006; Yamaguchi et al., 2006) and can contribute to the invasive behavior of tumor cells through release of chemokines (Negus et al., 1997; Brigati et al., 2002; Coussens and Werb, 2002; Pollard, 2004) or production of matrix-degrading proteases (Stetler-Stevenson et al., 1993; Sternlicht et al., 1999; Giraudo et al., 2004; Pollard, 2004; Boire et al., 2005). Work by Kitadai et al. (2006a, b) suggests that secretion of PDGF by tumor cells may also contribute to metastasis by acting on tumor PDGF-R positive stromal cells, which in turn may secrete growth factors supportive for the tumor. Similar mechanisms are seen in prostate and breast cancer cells that are capable of secreting endothelin-1 (ET-1). ET-1 binds the endothelin A receptor expressed in osteoblasts, resulting in their activation. The activated osteoblast produces growth factors essential for metastatic tumor growth in bone, thereby leading to bone metastases (Yin et al., 2003).

In summary, successful metastatic cells that arrive at secondary sites engage cells in the microenvironment as a means to modulate the secondary site and produce an environment conducive to metastatic cell survival. Recent data now suggest that the development of such an environment may occur in advance of the arrival of metastatic cells themselves via priming by bone marrow derived cells (Kaplan et al., 2005).

2.4.1. Cell–cell/microenvironment interactions—The ability of cells to effectively develop heterotypic interactions with other cells and to non-cellular elements in the microenvironment is pivotal in a metastatic tumor cell's ability to successfully adapt to its new surroundings. Integrins are critical players in forming these interactions, are known to mediate anchorage-independent growth (Eble and Haier, 2006), angiogenesis (Eble and

Haier, 2006), enhanced survival (Guo and Giancotti, 2004), and are thought to act as oncogenes and/or tumor-suppressor genes (Juliano and Varner, 1993; Hulleman and Boonstra, 2001). Briefly, integrins, which are frequently internalized and/or recycled (Caswell and Norman, 2006), consist of non-covalently associated α and β subunits with cytoplasmic, membrane, and extra-cellular domains (Hynes, 2002; Ramsay et al., 2007). Through extra-cellular matrix ligand binding, integrins can transduce signals to the cell and are also capable of responding to intracellular messages (Ramsay et al., 2007). Integrins may also have co-receptor functions that are adhesion-independent. Overexpression of β4 integrin, for example, can act as a signaling substrate for the HGF receptor C-met, resulting in enhanced anchorage-independent growth and tumorigenesis in nude mice (Bertotti et al., 2005, 2006).

Along with integrins, intercellular adhesion molecules (ICAMs), also play a significant role in mediating cellular interactions. ICAMs are members of the immunoglobulin superfamily and intimately involved in mediating cell–cell, cell–ECM, and immune interactions (Dustin et al., 1986; Roland et al., 2007). Because of their ability to influence cell–cell and cell–microenvironment interactions, it comes as no surprise that aberrant expression of these molecules is associated with both cancer development and metastatic progression. A summary of these associations with cancer is provided in Table 1.

2.4.2. Tumor stroma—The activated stroma of the tumor microenvironment consists of several components including growth factors, other secreted molecules, and host cells that are all thought to highly influence the behavior of tumor cells (reviewed in Liotta and Kohn (2001)). Of the host cells present in the tumor microenvironment, endothelial cells, macrophages (discussed above) and fibroblasts are established players in the metastatic process (Kalluri and Zeisberg, 2006). The cellular and non-cellular features that make up the tumor stroma are not only necessary for the metastatic behavior of cells but in fact have been shown to be sufficient. An activated “metastatic tumor stroma” is sufficient to convert non-metastatic cells to metastatic. The determinants of the metastatic tumor stroma” is in part generated by macrophages and fibroblast secretion of matrix metalloproteinases, which liberate a cascade of events that contribute to stromal activation and to chemokines (chemotactic proteins) and their associated receptors, which direct and modulate the cellular component of the stroma (Deryugina and Quigley, 2006). Table 2 lists specific features of the “metastatic stroma” and associations made with specific metastatic cancers.

2.4.3. Pre-metastatic niche—A novel hypothesis that extends our understanding of role of the stroma in the formation of metastasis is the pre-metastatic niche hypothesis (Kaplan et al., 2005). Kaplan et al. recently showed that tumor cell secreted factors were able to direct bone marrow derived VEGFR-1 $^{+}$ hematopoietic progenitor cells (HPCs) to future metastatic sites prior to the arrival of metastatic cells. Blocking the formation of the pre-metastatic niche prior to tumor development through the use of antibodies against VEGFR1 and VEGFR2 significantly inhibited metastasis (Kaplan et al., 2005, 2006). The HPCs of the pre-metastatic niche are thought to prime the pre-metastatic site in order to make it more favorable for incoming tumor cells. The migration of HPCs within the bone marrow is influenced by the interaction between the integrin VLA-4 and its ligand

fibronectin (Kaplan et al., 2006). Since upregulation of fibronectin occurs within the pre-metastatic niche and VEGFR-1^{+} HPCs express VLA-4, it is reasonable that the same interaction that influences migration of HPCs in bone marrow may also play a role in the adhesion of HPCs with the niche (Kaplan et al., 2005, 2006). The origins of the pre-metastatic niche in the bone marrow allows extension of a related hypothesis, that cancer cells may in fact transit to the bone marrow prior to their eventual spread to distant secondary sites. Similar to the pre-metastatic niche, the bone marrow microenvironment may provide a permissive environment for tumor cells (Kaplan et al., 2006). Indeed, several cancers metastasize to bone marrow in a CXCR4-dependent manner (Muller et al., 2001; Kaifi et al., 2005). Beyond protection, the bone marrow may in fact contribute to the metastatic phenotype of residing cells (Scadden, 2006). The reciprocal role of tumor cells on the bone marrow stroma is highlighted work from Nicola et al. (2003) who recently demonstrated that bone marrow stroma taken from breast cancer patients is significantly less adhesive towards tumor cells than normal bone marrow stroma. It is reasonable that this may make it easier for tumor cells to leave the niche after becoming ‘educated.’

2.5. Therapy

As discussed above, a better understanding of the molecular cross-talk between tumor cell and tumor microenvironment and how this affects the generally more agrgressive and chemotherapy-resistant biology of metastatic tumors is needed. However, despite new insights, metastasis still remains the overwhelming cause of death in cancer patients and new therapies are therefore needed that target the disease in a different way. An opportunity exists to improve outcomes for cancer patients by using our understanding the tumor cell (seed), the microenvironment (soil), and the molecular cross-talk between seed and soil.

Our understanding of the tumor seed and its stem-like features may predict the failure of treatments that target the tumor cell alone. Cancer cells with stem-like features (CSCs) may be best positioned to effectively resist the insult of many types of cancer therapy. While large fractions of tumor cells are sensitive, CSCs are thought to persist in a quiescent state only to recur at future times (Reya et al., 2001; Al-Hajj et al., 2004; Wicha et al., 2006). Targeting CSCs, or their protective niche may be the basis of more successful therapy (Yang and Wechsler-Reya, 2007). Exemplary of this, CSCs in brain tumors are thought to reside in “vascular niches” (regions rich in blood vessels) (Shen et al., 2004; Ramirez-Castillejo et al., 2006) that are often lined with endothelial cells that secrete stem cell survival and self-renewal factors. Calabrese et al. (2007) was able to show that by targeting these vascular niches with specific inhibitors in tumor-bearing mice, they were able to slow the growth rate of the tumor and significantly decrease the overall number of CSCs while having little effect on proliferation of most of the other tumor cells (Calabrese et al., 2007). Therefore, the addition of therapies that target CSCs in their niches to current treatment regimens may be valuable (Yang and Wechsler-Reya, 2007). An alternative means of targeting CSCs may include the use of differentiation agents. Compounds such as cyclopamine (hedgehog signaling) and imatinib (Wnt/β-catenin pathway) have been used to target pathways that are likely critical to a CSC’s ability to self-renew (Galmozzi et al., 2006; Li et al., 2007). It is tempting to speculate that the success of other differentiation inducers such as retinoic acid may be due to induction of differentiation in CSCs there by eliminating their ability to self-

renew (Li et al., 2007). Such therapies may be useful as part of long-term combination therapy strategies that target quiescent cancers with stem-like features.

The role of the tumor microenvironment in promoting tumor development and progression has been highlighted above. Therapies that now seek to modulate the tumor–host microenvironment and its components are feasible and should be considered (Langley and Fidler, 2007). For example, targeting macrophage/tumor cell interactions may be an attractive therapeutic target due to the multifactorial role macrophages play in regulating inflammation, angiogenesis, invasion, and the ECM. In support of this idea, macrophage knock-out mice exhibit a reduced rate of tumor growth and a dramatic decrease in metastases compared to litter-mate controls (Lin et al., 2001), while overexpression of the macrophage growth factor colony-stimulating-factor-1 accelerates the rate of tumor growth and metastasis (Lin et al., 2001). Additionally, a high density of tumor-associated macrophages is associated with a poorer prognosis in a large proportion of published studies (Lin et al., 2002). Targeting non-cellular features of the tumor stroma should also be considered as a treatment for metastasis. For example, induction of NO in the tumor stroma via expression of the NOS II expression machinery may be part of the mechanism associated with IFN-β and IFN-γ therapy, which has been shown to suppress metastasis in pancreatic adenocarcinoma (Wang et al., 2001). The hypoxic and acidic environment that is characteristic of tumor microenvironments may also be exploited to kill tumor cells. Bioreductive drugs, compounds that are only toxic under low oxygen conditions or reduced pH, such as tirapazamine have shown promising results when used in combination with other chemotherapeutic agents in clinical trials (Kovacs et al., 1999; Craighead et al., 2000; Xie and Huang, 2003). Similar strategies to target the hypoxic conditions of the tumor microenvironment may be considered as part of gene therapy using hypoxia-responsive promoters for gene expression (Xie and Huang, 2003). Other physiological conditions unique to the tumor microenvironment may limit current therapeutic approaches and may also be targets of novel treatments. Specifically, P-glycoprotein is a major component of the blood–brain barrier and other pharmacologic sanctuaries that are responsible for the poor penetration of chemotherapeutic agents (Cordon-Cardo et al., 1989). Thus, therapies that seek to modulate this protein may prove useful in the treatment of patients with CNS cancer or CNS metastasis.

Finally, targeting the cross-talk that occurs between the tumor cell and the microenvironment may over-ride the influence of the microenvironment on the metastatic phenotype. Classical targets of this cross-talk include VEGF, FGF, and PDGF. It is likely that the benefit of strategies that target these growth factors extends beyond the process of antiangiogenesis. (Kabbinavar et al., 2005; Sandler et al., 2006).

While much progress has been made to understand the biology of metastasis and metastatic lesions, many questions remain unanswered. The biology is complex with the cell’s fate being heavily influenced by the concepts introduced over 100 years ago in Paget’s seed and soil hypothesis. The phenotype of a metastatic cancer results from the tumor cell, the tumor microenvironment, and the interaction between tumor cell and its microenvironment. The process of metastasis is taxing (stressful). Successful metastatic cells are uniquely able to manage the stresses of metastasis by virtue of intrinsic features of the cell, the tumor

microenvironment that is in part activated by the tumor cell, and the ability of the tumor cell to successfully engage and interact with its microenvironment. As our understanding of this complex biology improves, new opportunities to target the tumor (seed), the tumor microenvironment (soil) and their interactions will emerge. It is hoped that these efforts will improve outcomes for patients with metastasis or at high risk for metastasis.

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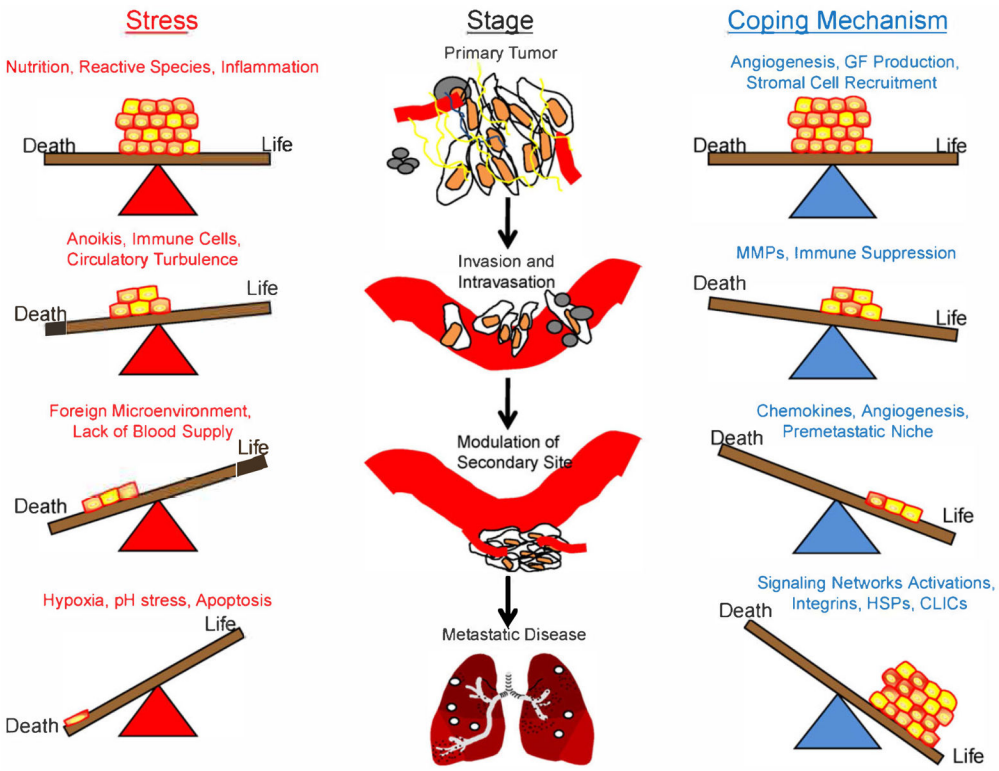

Fig. 1.

Tumor cells must resist stress in order to metastasize. Metastasis is thought to be a very inefficient process, in part, due to the number of stresses tumor cells must overcome in order to reach secondary sites and develop into gross metastatic lesions. Throughout each stage, tumor cells are confronted with various stresses, any of which may kill the cell. This results in a fragile balance between life and death for the cell. Only those tumor cells which can successfully manage the stress will survive. Depicted are examples of the various stresses tumor cells face during each stage of the metastatic process and some of the mechanisms the cell may use to deal with those stresses. Note that each stress and coping mechanism listed above are not exclusive to a particular stage of metastasis and likely apply to more than one of the stages. Cancer stem cells, those cells which are thought most able to resist the stresses of the metastatic cascade, are depicted in yellow. GF = growth factor; MMPs = matrix metalloproteinases; HSPs = heat shock proteins; CLICs = chloride intracellular ion channels.

Table 1

Heterotypic interactions between a tumor cell and its microenvironment (both cellular and non-cellular elements) are critical to the cell's ability to engage its surroundings in order to manage stress. Listed are some of the aberrantly expressed molecules that are thought to facilitate those interactions in specific cancers.

Molecule	Associated cancer	Reference
αvβ3	Melanoma	Nip et al. (1992)
α2β1, α3β1	Gastric carcinoma liver metastases	Ura et al. (1998)
αvβ3	Bone-residing metastases	Liapis et al. (1996), Byzova et al. (2000)
αvβ5	Colon carcinoma cells liver metastases	Enns et al. (2005)
α6β6	Colon carcinoma	Bates et al. (2005)
αvβ3	Colon carcinoma	Max et al. (1997)
ICAM-1	Melanoma, metastatic breast and liver carcinoma, gastric carcinoma	Natali et al. (1990, 1997), Sun et al. (1998), Rosette et al. (2005), Tachimori et al. (2005)
ICAM-2	Metastatic gastric carcinoma	Tanaka et al. (2004)
ICAM-5	Head and neck squamous carcinoma	Maruya et al. (2005)

Table 2

Tumor stroma consists of multiple components including growth factors, chemokines, various host cells, and other secreted molecules. “Activated” stroma is critical to the overall survival of the cancer and in many cases, is thought to enhance the tumor’s malignant capabilities. Listed below are some of the stromal components along with the cancer they have been associated with.

Stromal component	Associated cancer	Reference
Fibroblasts	Prostate, mammary	Olumi et al. (1999), Zeisberg et al. (2000), Tlsty (2001), Cunha et al. (2003), Bhowmick et al. (2004)
Macrophages	Mammary	Lin et al. (2001, 2002)
VEGF	Mammary, ovarian, gastric, colorectal kidney, bladder, colorectal, esophageal, thyroid, angiosarcoma, colon, melanoma, rhabdomyosarcoma	Ferrara and Davis-Smyth (1997), Fukumura et al. (1998), Brown et al. (1999), Belotti et al. (2008)
EGF	Breast, colorectal, ovarian, lung, head and neck	LeMaistre et al. (1994), Maihle et al. (2002), Arteaga (2006), Saldivar et al. (2006), Dickson and Deb (2007), Lafky et al. (2008)
PDGF	Lung, mammary, brain, bone metastases, prostate	Smits and Funa (1998), Paule (2005), Ingram and Bonner (2006), Roussidis et al. (2007)
IGF	Colon, lung, bone, breast, hepatocellular, liver, pancreatic, gastric, glioma	Ciampolillo et al. (2007), Monti et al. (2007), Samani et al. (2007), Sisci and Surmacz (2007)
TGF-β	Breast, colon oseophageal, gastric, hepatocellular, lung, pancreatic	Bierie and Moses (2006), Levy and Hill (2006)
MMP2	Ovarian metastases, hepatocellular, lung, brain metastases	Kenny et al. (2008), Wu et al. (2008), Guo et al. (2007), Mendes et al. (2005)
MMP3	Colorectal adenoma, lung, brain metastases	Lievre et al. (2006), Mendes et al. (2005), Fang et al. (2005)
MMP9	Lung metastases, mammary, ovarian, brain metastases	Martin et al. (2008), Belotti et al. (2008), Mendes et al. (2005)
CXCR4	Lung and bone metastases, mammary	Cardones et al. (2003), Murakami et al. (2002), Muller et al. (2001), Brown and Bicknell (2001), Knowles and Harris (2001), Luker and Luker (2006)
CCR4	Melanoma pulmonary metastses, lymphoma	Sokolowska-Wojdylo et al. (2005), Ishida et al. (2006)
CCR7	Gastric carcinoma lymph node metastases	Wiley et al. (2001), Mashino et al. (2002), Till et al. (2002), Ding et al. (2003), Takanami (2003), Takeuchi et al. (2004)
CCR9	Small intestinal metastases, prostate cancer	Letsch et al. (2004), Singh et al. (2004a)
CCR10	Melanoma	Murakami et al. (2003), Notohamiprodjo et al. (2005)