Cell

Cancer Metastasis: Building a Framework

Gaorav P. Gupta[1] and Joan Massagué[1,2,*]
[1]Cancer Biology and Genetics Program, and Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA
[2]Box 116, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA
*Contact: j-massague@ski.mskcc.org
DOI 10.1016/j.cell.2006.11.001

Metastasis occurs when genetically unstable cancer cells adapt to a tissue microenvironment that is distant from the primary tumor. This process involves both the selection of traits that are advantageous to cancer cells and the concomitant recruitment of traits in the tumor stroma that accommodate invasion by metastatic cells. Recent conceptual and technological advances promote our understanding of the origins and nature of cancer metastasis.

How tumors spread and kill their host organism remains an enigma, but not for lack of attention. For more than a century, cancer biologists have postulated that metastasis results from the interplay of wandering tumor cells with permissive target tissues. Yet, decades of scrutiny into the molecular bases of cancer have largely focused on what causes oncogenic transformation and the incipient emergence of tumors. By comparison, the study of how tumor cells take steps toward metastasis (that is, by altering their microenvironment, entering the circulation, and colonizing a distant organ) has received less attention. Progressively, however, the idea has emerged that tumors are more than just a mass of transformed cells. A renewed focus on the problem of metastasis is now apparent, and for good reason—metastasis remains the cause of 90% of deaths from solid tumors.

Several developments point toward progress. Recent work suggests that certain oncogenic events, such as evasion of growth suppression or of DNA-damage checkpoints, may also contribute to the evolution of tumors to the metastatic state because they create genomic instability. With increasing resolution, the genetic and epigenetic aberrations in tumors and their surrounding stroma are being profiled genomewide in both animal models and clinical samples. New evidence points at the engagement of cellular accomplices from the stroma to aid in tumor-cell survival and parasitic dominance at distant organ sites. Molecular mediators of tumor-cell homing to and colonization of specific organ sites are also beginning to emerge. Recent technological advances allow validation of these new findings through the analysis of clinical samples. Taking stock of these developments may help in the creation of a roadmap to guide future work.

A Problem of Evolution

An underlying concept in our analysis is that metastasis emerges from the somatic evolution of a genetically diversified cancer-cell population under the selective pressures of an environment that imposes tight rules on cell behavior. In essence, this explains why millions of cells might be released by a tumor into the circulation every day, but only a tiny minority of these cells will colonize a distant organ. The utter inefficiency of the metastatic process implies that healthy tissues display a marked hostility toward invading tumor cells. This is not surprising. In a highly evolved organism, homeostatic mechanisms ensure that order is maintained in its tissues. To achieve metastasis, cancer cells must therefore evade or co-opt multiple rules and barriers that were refined over hundreds of millions of years of organismal evolution. Thus, metastasis is akin to an evolutionary process that involves selection of genetically heterogeneous lineages of cancer cells within the ecosystem of an organism.

Several discrete steps are discernable in the biological cascade of metastasis: loss of cellular adhesion, increased motility and invasiveness, entry and survival in the circulation, exit into new tissue, and eventual colonization of a distant site (Chambers et al., 2002; Fidler, 2003). Seminal work using experimental assays for metastasis demonstrated that rare clones within malignant cell populations were endowed with several of these metastasis-promoting functions (Fidler, 2003). The implication was that cells that comprise a metastatic lesion were descendants of an exceedingly rare cell from the primary tumor that stochastically expressed many, if not all, of the genes necessary for successful execution of the metastatic cascade (Fidler, 2003).

Recent advances in the molecular profiling of cancer using genomic-level approaches have revealed genes whose expression in primary tumors correlates strongly with the likelihood of metastatic recurrence (Weigelt et al., 2005). These observations have also prompted a reconsideration of how, where, and when cancer cells acquire genes of relevance to metastasis and have raised the possibility that cells with metastatic potential may not be as rare in primary tumors as was originally believed (Bernards and Weinberg, 2002). Furthermore, recent evidence underscores the profound impact that the transformed cell of origin has on the metastatic course of a tumor—an important concept that conventional models for the selective evolution of cancers did not fully appreciate.

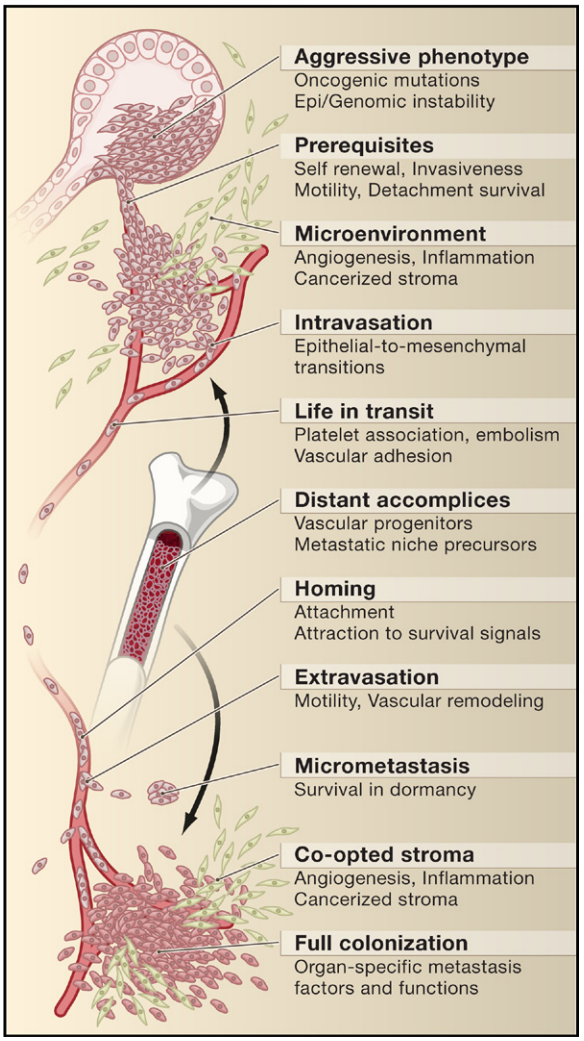

Figure 1. Stages of Metastatic Progression

Metastasis proceeds through the progressive acquisition of traits that allow malignant cells originating in one organ to disseminate and colonize a secondary site. Although these traits are depicted as part of a contiguous biological sequence, their acquisition during metastatic progression need not follow this particular order. Although in some cases several factors may be necessary to implement a single step in this cascade, other mediators of metastasis may facilitate execution of multiple stages simultaneously. Similarly, the specific steps of this sequence that are rate limiting for metastatic progression may also vary from one tumor to the next.

Although developments such as these reflect the complexity of cancer progression, they do not detract from the idea that metastatic cells must overcome numerous physical obstacles barring metastasis. The common biological challenges posed by these barriers suggest that there might be recurrent themes for metastatic progression, just as there are for primary tumor formation (Figure 1). In this way, cancer may progress as a disease of genetically heterogeneous cell populations driven to evolve by sequential environmental pressures.

The Early Origin of Cellular Heterogeneity

Evolutionary processes require a source of heterogeneity within the population, from which advantageous traits can be selected. In the context of a tumor, this heterogeneity is amply supplied by the intrinsic instability of cancer genomes in the form of DNA mutations, chromosomal rearrangements, and epigenetic alterations. Evidence that highly metastatic clones from tumor-cell populations had a higher rate of genetic mutability than nonmetastatic clones from the same tumor provided an early link between metastasis and genetic instability (Fidler, 2003).

Major alterations in genomic DNA were once viewed as an exclusive trait of advanced cancers. However, it is now recognized that DNA damage and genomic instability are underlying features of human cancer from the earliest stages of tumorigenesis. Damage to genomic DNA is evident even in apparently normal cells and becomes amplified as tumors emerge (Bartkova et al., 2005; Feinberg et al., 2006; Gorgoulis et al., 2005). Genomic instability may be directly driven by mutations leading to tumor initiation that were once thought to cause abnormal cell proliferation but not much else. For example, inactivation of the cell cycle suppressor Retinoblastoma (Rb) alters the expression of the mitotic checkpoint regulator Mad2, which fosters aneuploidy (Hernando et al., 2004). Hyperactive mediators of oncogenic signaling such as Akt can attenuate the DNA-damage checkpoint response by disabling damage sensors such as the kinase Chk1 (Puc et al., 2005). Telomeric crisis may wreak havoc on the genomes of cancer cells, producing a myriad of traits associated with tumor progression (Maser and DePinho, 2002). Moreover, epigenetic plasticity must be recognized as an important source of cancer-cell heterogeneity (Baylin and Ohm, 2006; Feinberg et al., 2006). As a recent example, ectopic overexpression of the polycomb group protein EZH2, which results in alterations in chromatin remodeling, correlates with metastasis and poor overall survival in prostate cancer patients (Varambally et al., 2002).

Pressures that Select for an Aggressive Phenotype

The inappropriate proliferation of cells harboring oncogenic lesions is challenged by multiple layers of mechanisms that suppress tumor formation (Figure 2). Several of these barriers are cell intrinsic (such as the genotoxic stress induced by oncogenes, the expression of growth inhibitory, apoptotic and senescence pathways, and telomere attrition). Evasion of these tumor suppressive pathways is a hallmark of primary tumors (Hanahan and Weinberg, 2000). However, an entirely distinct class of pressures comes from sources that are extrinsic to the cancerous cells. Factors in the tumor microenvironment that limit tumor progression include extracellular matrix components, basement membranes, reactive oxygen species, the limited availability of nutrients and oxygen, and attack by the immune system (Figure 2). How tumors cells respond to these external cues influences,

sometimes in dramatic fashion, their metastatic potential. An example is provided by the cellular response to hypoxia, which is emerging as a major player that shapes the aggressiveness of primary tumors.

In tumors, hypoxia is a strong selective pressure that promotes the outgrowth of malignant cells with a diminished susceptibility to undergo apoptosis. The cellular response to low oxygen tension involves the stabilization of a hypoxia inducible factor-1 (HIF-1) transcriptional complex that activates genes that promote angiogenesis, anaerobic metabolism, cell survival, and invasion (Harris, 2002). Tumors that exhibit abundant HIF-1 stabilization have a greater likelihood of developing metastatic relapse and correlate with a shorter survival time (Semenza, 2003). Accordingly, by analyzing global transcript levels an "epithelial cell hypoxia signature" has been established that is an independent predictor of metastatic risk for both breast and ovarian carcinomas (Chi et al., 2006). A subset of HIF-1 target genes may act as mediators of metastatic progression. HIF-1 induces the expression of the chemokine receptor CXCR4 in renal cell carcinoma cells, which may promote organ-specific metastatic dissemination (Staller et al., 2003). A recent study implicates lysyl oxidase (LOX) as a HIF-1 target that mediates metastasis of human breast cancer cells in a mouse model and correlates with poor overall survival among estrogen receptor-negative breast cancer patients (Erler et al., 2006). LOX is required for the maturation of newly synthesized collagen fibrils and may promote metastasis through changes in focal adhesion kinase activity. Hypoxia can also induce expression of Met, thereby facilitating tumor cell invasion mediated by HGF (the ligand for Met) (Pennacchietti et al., 2003).

Other aspects of the microenvironment may also drive the selective evolution of primary tumors. For instance, reactive species of nitrogen and oxygen, which are generated by both infiltrating inflammatory cells and rapidly proliferating tumor cells, can contribute to the genomic instability of cancer cells and promote the expression of genes that facilitate metastasis (Hussain et al., 2003). Tumors also exert different physical pressures than well-organized tissues. For example, tensional forces on mammary epithelial cells during tumorigenesis may result in clustering of mechanotransducing integrins and subsequent downstream activation of ERK and Rho-GTPase (Paszek et al., 2005). These signaling events promote tumor-cell proliferation and disrupt tissue polarity.

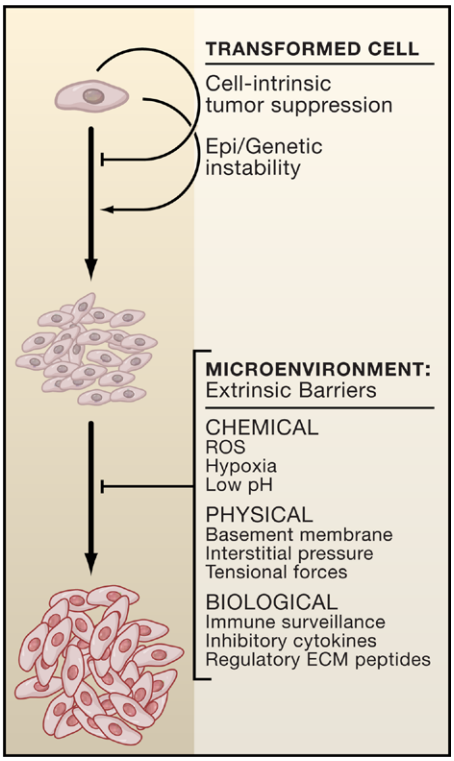

Figure 2. Pressures that Drive Selection for Metastatic Traits

Cell-intrinsic mechanisms limit the aberrant hyperproliferation of normal cells. Bypass of these cellular restraints, in part fueled by genomic and epigenomic instabilities, is a hallmark of cancer. The local microenvironment provides extrinsic barriers that are evolutionarily conserved to preserve normal tissue structure and function. These barriers can be broadly classified as chemical, physical, or biological in nature. Examples for each category are provided. These extrinsic barriers limit the outgrowth of tumors at the primary site, but a related set of barriers also challenges the intrusion of disseminated cancer cells into a secondary organ. As tumors evolve, these pressures drive the selection for traits that enable cancerous cells to bypass them. Tumors with limited cellular heterogeneity may be unable to overcome these pressures and may spontaneously regress or subsist in balance with these tumor suppressive forces. Alternatively, in tumors containing a high degree of cellular heterogeneity, aggressive cellular subpopulations that can resist, co-opt, or overcome these barriers may dominate the cancer, rendering it primed for metastatic progression.

Prerequisites for Metastasis

Tumor-Initiating Capacity

Normal cells constitute lineages that extend from stem cells to terminally differentiated progeny. Stem cells have the capacity to divide with at least one daughter retaining the phenotype of the mother. Similarly, the long-term tumorigenic potential of some tumors may rely on a small proportion of malignant cells endowed with a similar capacity to indefinitely self renew. These tumor-initiating cells are sometimes referred to as cancer stem cells (Pardal et al., 2003). As was originally demonstrated for hematological malignancies (Bonnet and Dick, 1997), solid malignancies of the breast and brain have recently been shown to contain cells with such tumor-initiating capacity (Al-Hajj et al., 2003; Singh et al., 2004). When isolated, these cells were capable of giving rise to all other transformed cellular phenotypes (as defined by cell surface markers) observed in the original tumor. Additionally, they were capable of initiating secondary tumors from very low numbers of transplanted cells (a surrogate for self-renewal activity). However, the idea that self renewal in solid tumors is a property of only a tiny cell subpopulation in a tumor mass is currently supported by limited evidence. Moreover, if self-renewing tumor cells are the only cells capable of generating secondary growths, then one might expect that the prevalence of tumor-initiating cells in a tumor would reflect the overall proclivity for metastatic recurrence. This, however, has yet to be shown. Regardless of the relative abundance of self-renewing

cells in primary tumors, a capacity for tumor initiation is a must for the reestablishment of the tumor by a few surviving cells in a distant metastatic location.

What then are the molecular mechanisms that bestow upon cancer cells the ability to initiate tumors? Some answers to this question are beginning to emerge. The polycomb family protein Bmi-1, a transcriptional repressor, mediates both self renewal in normal hematopoietic and neural stem cells, as well as tumor-initiating capacity among "leukemic stem cells" (Valk-Lingbeek et al., 2004). The Wnt/β-catenin, Hedgehog, and Notch signaling pathways are also implicated in stimulating self renewal in both normal stem cells and malignant cells (Beachy et al., 2004; Radtke and Clevers, 2005). Genetic alterations that activate otherwise normal self-renewing mechanisms may enhance metastatic efficiency. For example, overexpression of the transcriptional effector of Hedgehog signaling, Gli1, in rat prostatic carcinoma cells rendered them aggressively metastatic to the lungs (Karhadkar et al., 2004). For this reason, targeting these pathways in cancer raises concerns about possible toxicity to normal stem cells. However, the recent finding that hyperactive Akt signaling is necessary for leukemia-initiating capacity but is a deterrent of hematopoietic stem-cell renewal exemplifies a promising opportunity for targeting malignant self renewal while sparing host stem cells (Yilmaz et al., 2006).

Altered Cellular Adhesions

Compared to normal epithelia, carcinoma cells almost invariably show diminished intercellular adhesiveness (Cavallaro and Christofori, 2004). In many instances, epithelial tumors lose E-cadherin-mediated adhesions as they progress toward malignancy. Documented mechanisms for E-cadherin loss in tumors include inactivating mutations that predispose to gastric cancer, epigenetic silencing, proteolytic cleavage, and proteosomal degradation (Cavallaro and Christofori, 2004). Additionally, E-cadherin expression can be repressed as part of a broader program resembling an epithelial-to-mesenchymal transition (EMT). EMT can occur in cancer cells upon activation of specific transcription factors (such as Snail, Twist, and Slug), many of which are involved in EMT during embryogenesis.

Integrins are also emerging as important mediators of the malignant phenotype during oncogenic transformation (Guo and Giancotti, 2004). In particular, the $\alpha_6\beta_4$ integrin, which binds to the extracellular matrix protein laminin, forms signaling complexes with oncogenic receptor tyrosine kinases, including Met, EGFR, and Her2 (Guo and Giancotti, 2004). $\alpha_V\beta_3$ and $\alpha3\beta_1$ integrins have also been implicated in later stages of metastasis, specifically during adhesion of circulating tumor cells to the vasculature (Felding-Habermann et al., 2001; Wang et al., 2004). Thus, diverse alterations in adhesive properties allow cancer cells to disobey the rules of tissue architecture and to advance in their malignant progression.

Cell Motility

Metastasis fundamentally involves the movement of cells from one site to another. A molecular depiction of cell migration in in vitro models has emerged, which involves dynamic cytoskeletal changes, cell-matrix interactions, localized proteolysis, actin-myosin contractions, and focal contact disassembly (Friedl and Wolf, 2003). Nodes of regulation include small GTPases (such as Rho, cdc42, and Rac), integrin-containing focal adhesion assembly and disassembly, secreted and plasma membrane-tethered proteases, and the actomyosin contractile machinery. Growth-factor signaling, such as that mediated by HGF through the Met receptor, can modulate many of these activities either directly or indirectly (Liotta and Kohn, 2001). Several recent studies have implicated components of this cell motility machinery in metastatic progression. Comparative genomic analysis of in vivo selected melanoma cell lines in mice revealed a role for RhoC in lung metastasis (Clark et al., 2000). Comparative oncogenomic analysis of a mouse model of melanoma has shown that an increase in copy number and overexpression of Nedd9, a focal adhesion kinase (FAK) adaptor protein, fosters cell motility and invasion (Kim et al., 2006). Nedd9 was also independently identified as one of a set of genes that mediate the metastasis of breast cancer to the lungs (Minn et al., 2005a). Podoplanin, a mucin-like transmembrane glycoprotein that is expressed at the invasive front of many human malignancies, enhances cellular invasiveness independently of E-cadherin loss, perhaps by regulating the cytoskeletal anchoring protein Ezrin (Wicki et al., 2006).

Recent advances in intravital imaging of primary tumors in mice have uncovered properties of cancer-cell movement in vivo that differ from those that were originally observed in vitro. Malignant cells in breast cancers move much faster than similarly transformed cells on tissue-culture plastic or in reconstituted extracellular matrix (Condeelis and Segall, 2003). The faster mobility in vivo appears to involve the ability of malignant cancer cells to rapidly track along collagenous extracellular matrix fibers. Intriguingly, acquisition of this motile property coincides with manifestation of the metastatic phenotype (Condeelis and Segall, 2003). Confirming the relevance of this prometastatic motile behavior to human cancer and dissecting the pathways that underlie it are important avenues for future research.

Resistance to Extracellular Death Signals

Although evasion of apoptosis is a hallmark of tumor cells, it is possible that progression toward metastasis requires a further defense against microenvironmental death stimuli. Nutrient deprivation and hypoxia, alterations in extracellular adhesions, changes in cell shape during invasion, and exposure to novel stromal microenvironments can all trigger cell death. Ectopic overexpression of potent anti-apoptotic effectors, such as BCL2, BCL-X_L, and XIAP, in cancer cells can make them highly resistant to death stimuli, which has been shown to enhance the efficiency of metastasis in numerous experimental models (Mehlen and Puisieux, 2006). Alternatively, loss of caspase 8 expression, an apoptotic initiator caspase activated downstream of unligated integrins, can also facilitate invasion and metastasis by

making tumor cells more resistant to stress from loss of adhesion (Stupack et al., 2006). Pediatric neuroblastomas with evidence for genomic loss at the caspase 8 locus are commonly associated with a poor overall prognosis (Brodeur, 2003).

Disruption of the Basement Membrane and Extracellular Matrix

Basement membranes that underlie epithelial and endothelial cell layers are a dense meshwork composed of several glycoproteins and proteoglycans (such as type IV collagen, laminin, perlecan). A well-organized basement membrane is an integral contributor to epithelial structure, providing both a physical boundary as well as a signaling substrate to orient cells through integrin-based adhesions. For epithelial tumors in an incipient state, the basement membrane acts as a barrier to the invasion of transformed cells into the subjacent stroma. Tumor cells that are able to proteolytically disrupt the basement membrane can progress to overt malignancy and metastasis.

The activity of extracellular matrix proteases is normally under tight control through specific localization, autoinhibition, and secreted tissue inhibitors (reviewed in Egeblad and Werb [2002] and Liotta and Kohn [2001]). Cancerous cells use diverse mechanisms to disrupt this tight regulation and unleash proteolytic activities on the basement membrane and interstitial extracellular matrices. In addition to facilitating tumor invasion, extracellular proteases may generate a diverse array of bioactive cleaved peptides. These products can modulate migration, cancer-cell proliferation and survival, and tumor angiogenesis. Adding complexity, some of the pleiotropic effects of matrix metalloproteinases (MMPs) may actually antagonize tumor growth (Overall and Kleifeld, 2006). The physiological importance of MMPs is evident by the extensive joint disorders caused by MMP inhibitors in clinical trials, which has so far deterred the effective use of these agents in anticancer therapy (Coussens et al., 2002). Segregating the pro- versus antimetastatic component activities of extracellular proteases during different stages of cancer progression will be instrumental in designing a new generation of specific protease inhibitors that may be more clinically effective than the initial unsuccessful efforts.

Beyond the Basement Membrane: Enemies and Accomplices

Progression of invasive carcinomas requires collusion between tumor cells and multiple nontransformed cell types residing in (or being recruited to) the tumor stroma (Figure 3). Indeed, several histopathological markers of stromal cell cooption in tumors, such as fibrosis, leukocytic infiltration, angiogenesis, and lymphangiogenesis, are frequently correlated with an increased likelihood of metastatic relapse. It could be that tumor cells that can convert reactive stromal infiltrates from preservers of homeostasis into accomplices in malignancy earn a selective advantage in the primary tumor and at sites of

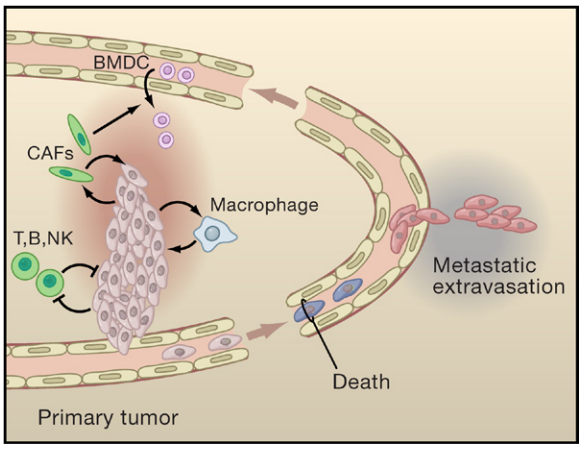

Figure 3. Distinct Fates for Disseminated Cancer Cells

Advanced malignancies are frequently coinhabited by different stromal cell types. The tumor-suppressive activities of lymphocytes (T, B, and NK cells) are kept in check in part through the release of immunosuppressive cytokines (e.g., TGFβ, IL-10, and IL-23). Carcinoma-associated fibroblasts (CAFs) can secrete factors that promote tumor-cell growth and invasiveness and also promote angiogenesis through recruitment of endothelial precursor cells (EPCs) from circulation. Activated macrophages are also recruited to tumors and release many protumorigenic growth factors. Additionally, there is evidence that macrophages may comigrate with cancer cells within tumors through a paracrine growth-factor loop. Once cancer cells have invaded the blood stream, many of them will die from stresses associated with circulatory passage. Those that are able to resist death may attach to capillaries within a secondary organ through adhesion receptors, which may in addition provide survival signals. Subsequently, disseminated cancer cells may or may not thrive at the secondary site, with or without metastatic extravasation.

metastasis. An area of intense interest, the topic of stromal contributions to cancer, has been covered by several recent reviews (Condeelis and Pollard, 2006; de Visser et al., 2006; Kalluri and Zeisberg, 2006). Here, we will discuss specific examples of this crosstalk that highlight its potential influence on the evolution of cancer toward metastatic competency.

As tumors evolve, are there driving forces that select for accommodating traits in the cancer-associated stroma? Heterotypic interactions between epithelial and mesenchymal cells are essential for proper morphogenesis during embryo development. For decades, cancer biologists have suspected that these same interactions could enhance the malignant phenotype of carcinomas. Targeted disruption of transforming growth-factor β (TGFβ) signaling in fibroblasts can induce carcinomas of the forestomach and prostate in mice (Bhowmick et al., 2004). Although this phenomenon has yet to be observed in human cancer, it provides a striking example of the malignant potential that arises from the disruption of tumor-suppressive crosstalk. Studies using a transgenic mouse model for prostate cancer provide another intriguing example (Hill et al., 2005). Crossing these tumor-prone mice to mice heterozygous for p53 led to a loss of heterozygosity and a growth advantage in tumor-associated fibroblasts before any such events

were evident in the tumor epithelium. Mutations in p53 have also been observed in the cancer-associated stroma of human breast tumors (Kurose et al., 2002). Other studies have described PTEN mutations and epigenetic alterations in cancer-associated stroma (Hu et al., 2005; Kurose et al., 2002). However, further work will be necessary to establish whether loss of p53 and PTEN functions in the stroma provide an advantage to the adjacent tumor cells that goes beyond effects on tumor-associated stromal cells.

Does the recruitment of tumor-promoting mesenchyme confer properties to an evolving cancer that promote metastasis? Evidence suggests that this may be the case. A gene expression signature of fibroblast activation in vitro was able to segregate primary breast tumors according to their likelihood of giving rise to metastases in patients (Chang et al., 2004). This may simply reflect the reaction of the stroma to an aggressive tumor, or it might indicate that a tumor's aggressiveness is influenced by the composition and activity of its stromal infiltrate. Indeed, how cancer-associated fibroblasts contribute to metastatic progression is under intense investigation. One piece of the puzzle involves the production of the chemokine CXCL12 by breast cancer-associated fibroblasts (Allinen et al., 2004; Orimo et al., 2005). Upon examination in xenograft and in vitro models, CXCL12 augmented the proliferation and migratory activity of tumor cells while concomitantly facilitating angiogenesis in developing tumors. The latter was partly mediated by the recruitment of circulating endothelial progenitor cells expressing CXCR4, which is the receptor for CXCL12 (Figure 3) (Orimo et al., 2005). Insights like this into mesenchymal facilitation of metastatic progression may identify promising new approaches for cancer therapy.

Other cell types that are frequently present in tumors include leukocytes and lymphocytes that may be recruited there as a physiological response to tissue injury. Natural killer cells, antigen presenting cells, and different subclasses of T and B lymphocytes are in charge of the immune attack and are thus in general tumor suppressive. However, this potent response is frequently blunted during the progression of neoplasms by the tumor-derived overexpression of immunosuppressive cytokines such as TGF-β, interleukin-10, and interleukin-23 (Gorelik and Flavell, 2002; Langowski et al., 2006; Zou, 2005). Furthermore, tumor cells may not provide the costimulatory signals necessary to elicit a robust immune response, such as those that neutralize the autoinhibitory activity of cytotoxic-T lymphocyte antigen-4, CTLA-4 (Chambers et al., 2001).

Specific contributions of the innate immune response to tumor development and progression are complex and have been the subject of several recent reviews (Condeelis and Pollard, 2006; de Visser et al., 2006). Overall, the cells and cytokines that mediate chronic inflammation facilitate both tumor initiation and metastatic progression. Some of the metastasis-promoting effects may impinge on cytokine-mediated activation of the nuclear factor kappa B (NF-κB) pathway in tumor cells (Karin, 2006). Additionally, the synthesis of prostaglandins by inflammatory cells expressing the inducible cyclooxygenase 2 (COX-2) may also participate in metastatic progression (Dannenberg and Subbaramaiah, 2003; Minn et al., 2005a). At the cellular level, tumors that have been infiltrated by activated macrophages typically follow an aggressive course of disease (Condeelis and Pollard, 2006). Attracted to regions of hypoxia and necrosis, tumor-associated macrophages potently induce angiogenesis by secreting copious amounts of vasoactive factors (e.g., VEGF, IL-8, and PGE_2), as well as proteases that enhance their bioactivity (e.g., MMP-9 and uPA) (Condeelis and Pollard, 2006). Additionally, macrophages release several growth factors (e.g., EGF, PDGF, and HGF) that may facilitate tumor-cell proliferation, survival, and invasion during cancer progression (Condeelis and Pollard, 2006). Mice with defects in the macrophage lineage due to mutations in CSF-1 rarely develop lung metastases from aggressive polyoma middle T-driven mammary tumors (Condeelis and Pollard, 2006). Modulation of the metastatic phenotype through targeted intervention of specific branches of the innate immune response remains a promising future area of cancer therapy.

Highways to Distant Organs

In order to metastasize, cancer cells must invade tumor-associated vasculature to gain access to distant sites in the body. This is facilitated by the need of developing tumors to establish neo-vasculature in order to grow beyond the diffusion limit of preexisting blood vessels (Hanahan and Folkman, 1996). The acquisition of this angiogenic phenotype—termed the "angiogenic switch"—represents a vital step in the evolution of solid tumors (Hanahan and Folkman, 1996). This event occurs partly through induced outgrowth of the preexisting vasculature and partly through de novo recruitment of vascular cell precursors from the circulation. Lymphangiogenesis is also observed in advanced primary cancers. This results in a tortuous network of lymphatic vessels designed to collect interstitial fluid effusions and carry them to lymph nodes and subsequently into hematogenous circulation (Alitalo et al., 2005). Perhaps because lymphatic vessels are more leaky in their design than blood vessels—owing to the lack of tight intercellular junctions between lymphatic endothelial cells—the presence of lymph-node metastasis often represents an early prognostic indicator of tumor invasiveness and metastatic dissemination in several types of carcinoma and in melanoma (Alitalo et al., 2005). However, other metastatic malignancies, such as sarcomas, are notorious for metastasizing to distant sites without any prior evidence of local spread to regional lymph nodes. Regardless, it is thought that access to all organs of the body (lymph nodes excluded) is predominantly through the hematogenous circulation.

The molecular mechanisms controlling intravasation (the penetration of the blood vessels by invading tumor cells) remain to be defined. In a recent study of genes involved in lung metastasis of a mouse mammary tumor cell line, enhanced expression of Twist—a transcription factor that promotes EMT transitions—was found to favor metastasis due to its ability to augment the rate of hematogenous intravasation (Yang et al., 2004). It remains unknown whether this effect was due to the acquisition of specific biological functions that enabled breach of the endothelial vasculature or was an indirect consequence of the enhanced motility of cancer cells with mesenchymal attributes. It is possible that once cancer cells become highly motile within primary tumors, they are naturally attracted to blood vessels due to chemoattractive gradients and extracellular matrix tracks emanating from (or terminating) there. Indeed, this was observed in the intravital imaging studies of experimental mammary carcinomas (Condeelis and Segall, 2003). Technological advances that enable the isolation and genomic analysis of circulating cancer cells from patients and in experimental tumor models may yield novel insights into the molecular prerequisites of malignant intravasation.

Life in Transit

Once malignant cells have invaded this circulatory compartment, they attain ready access to virtually all organs of the body (Figure 3). However, they must be able to survive several stresses, including physical damage from hemodynamic shear forces, and immune-mediated killing. Recent advances have begun to make headway into the mechanisms that allow metastatic cells to evade these perils.

Circulating tumor cells may promote their survival by co-opting blood platelets, using them as shields. Clinically observed for well over a century, malignancies have a tendency to induce a hypercoagulable state in their hosts (Nash et al., 2002). Histopathological analysis of early-stage hematogenous metastases in humans frequently reveals the coexistence of thrombosis, with abundant fibrin deposition (Ruiter et al., 2001). Disrupting the interaction between tumor cells and platelets in experimental models has validated this relationship as causal for metastasis to multiple target organs (Nash et al., 2002). Consequently, tumor emboli are believed to possess greater metastatic potential than naked tumor cells, owing at least in part to their resistance to immune-mediated mechanisms of clearance and to physical hemodynamic forces (Nash et al., 2002). Understanding the detailed mechanisms that underlie tumor-cell and platelet adhesion and interaction, as well as selective ways of inhibiting them without disrupting physiological hemostasis, may translate into promising antimetastasis therapies when initiated early in the course of disease progression.

Consideration has also been given to mechanisms that may allow evasion of cell death that is induced by the loss of adhesive supports (referred to as anoikis). The brain-derived neurotrophic factor (BDNF) receptor trkB conferred resistance to anoikis to immortalized cells in vitro and increased the metastatic activity of a rat intestinal epithelial cell line (Douma et al., 2004). However, the relevance of anoikis in the process of metastasis remains uncertain. In humans, it may take mere minutes for a malignant cell departing from a primary tumor to encounter a capillary bed and adhere to the vascular wall. If the time that circulating tumor cells spend devoid of adhesion is so short, anoikis may not be a very significant impediment during the physiological progression of metastasis. As mentioned earlier, resistance to death from detachment may be more relevant at earlier stages of the metastatic cascade.

Rapid mechanical lodging in capillaries and association with platelets are likely a prevalent form of tumor cell entrapment in distant organs. However, it is also possible that the initial homing of disseminated cancer cells to a secondary organ involves adhesive interactions between cell-surface receptors expressed on malignant cells and their cognate ligands expressed in various target sites for metastasis. Integrin receptors have been proposed to participate in such homing interactions. For example, $\alpha 3\beta 1$ integrins expressed on circulating tumor cells have been shown to bind to laminin-5 within exposed regions of the vascular basement membrane during lung metastasis (Wang et al., 2004). Using a phage display screening approach, a novel adhesion receptor was identified and named metadherin, which was found to be expressed by metastatic breast cancer cells and binds to an as of yet uncharacterized ligand expressed selectively on lung-capillary endothelial cells (Brown and Ruoslahti, 2004). In addition to adhesion receptors, chemokines and their receptors have also been implicated in metastatic-cell homing to target tissues. For example, CXCR4 expression in breast cancer cells was shown to be important for metastasis to CXCL12-rich tissues, such as the lungs (Muller et al., 2001). These and other receptor-ligand pairs may account for some of the heterogeneity in target-tissue homing exhibited during metastatic dissemination of different primary tumors.

Getting out: Extravasation

Having invaded and endured the circulation, metastatic cells must at some point escape once again, but this time out of the endothelial vasculature and into a target tissue in a process called extravasation (Figure 3). Exactly when this event occurs in the cascade of metastasis may vary from tumor to tumor. In some cases, considerable growth within the intravascular space may occur until the lesion physically bursts through the limiting surrounding vasculature (Al-Mehdi et al., 2000). The cytoskeletal anchoring protein ezrin may facilitate this escape in metastatic osteosarcoma cells. Inhibiting ezrin's expression in these cells resulted in higher rates of cancer-cell

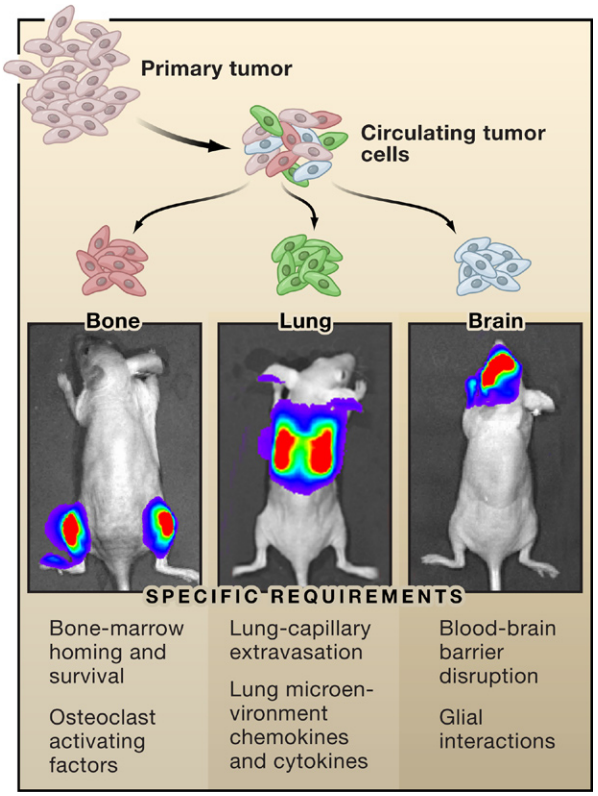

Figure 4. Patterns of Metastatic Colonization
Circulating tumor cells are confronted by nonreceptive secondary organ sites. Each of these sites exhibits properties that make it distinct from the organ from which the malignant cells disseminated. To successfully colonize a secondary site, metastatic cells must adopt a signaling vocabulary that is appropriate for the extrinsic microenvironment. Because different organs will place different demands on the invading tumor cells, there will be selection for distinct mediators of metastatic colonization. These concepts have been demonstrated through analysis of a heterogeneous population of breast-cancer cells derived from pleural effusions in a patient with widely metastatic breast cancer. In vivo selection for specific metastatic tropisms using immunocompromised mice has yielded cellular subpopulations with highly enriched metastatic activity to the brain, lungs, and bone. Metastatic activity was visualized using bioluminescent imaging. As expected, the genes that mediate these aggressive site-specific metastatic activities are largely distinct.

death prior to metastatic extravasation into the lung parenchyma (Khanna et al., 2004).

Are there signals emanating from metastatic cells that actively induce changes in the vascular permeability of blood vessels in target organs? VEGF—initially identified as a potent vascular permeability factor—is one prime candidate (Weis and Cheresh, 2005). The activation of Src family kinases in endothelial cells exposed to VEGF induces disruptions in endothelial cell junctions, which can facilitate metastatic extravasation. Consistent with this, Src knockout mice were protected from lung metastatic colonization by VEGF-secreting cancer cells (Criscuoli et al., 2005). Further exploration of molecular players mediating this potentially rate-limiting step of metastatic progression will determine if it occurs within a therapeutically susceptible time frame.

Patterns of Colonization

The organ distribution of full-blown metastases from a primary tumor is not random (Figure 4). After analyzing secondary cancer outgrowths in a series of autopsies for breast-cancer victims, Stephen Paget proposed that disseminated cancer cells, or "seeds," would only colonize organ microenvironments, or "soils," that were compatible with their growth (Paget, 1889). Clinical observation of cancer patients supports the notion that circulatory patterns alone provide only a partial explanation for preferred sites of metastasis (Fidler, 2003). For example, systemic breast cancer frequently metastasizes to the lungs, bones, liver, and brain—most of which do not have a direct circulatory connection to breast tissue. Advanced prostate cancer has a more selective pattern of metastatic recurrence, with bone being the predominant site, whereas visceral organs such as the lungs or liver are much more rarely involved. Uveal melanomas metastasize with astonishing specificity to the liver—and sarcomas to the lungs.

One prediction of Paget's seed-and-soil hypothesis is that metastatic cells will only colonize compatible target tissues, even if they are artificially targeted in large numbers to inhospitable sites. A demonstration of this phenomenon in humans was serendipitously obtained in ovarian-cancer patients that received palliative peritoneovenous shunting of their ascites fluid into the jugular vein (Tarin et al., 1984). Intended to relieve the pain and discomfort of malignant ascites without causing metabolic imbalances that arise from direct removal of large quantities of peritoneal fluid, this procedure inadvertently allowed the release of millions of metastatic cancer cells directly into the venous circulation of cancer patients over the remainder of their lives. Astonishingly, the majority of patients did not develop disseminated metastases, sometimes even after two years of continuous vascular shunting. Furthermore, even when metastases were observed after autopsy, they were frequently indolent growths.

What are the molecular and cellular determinants of unique metastatic tropisms? Recent advances have brought us closer to understanding the molecular and cellular bases for this important aspect of metastasis. It is now appreciated that at least two classes of determinants affect site-specific metastatic outgrowth. First, there must be an initiation of a viable premetastatic niche within the target organ—one that facilitates the initial survival of extravasated tumor cells in a nonreceptive target organ. Subsequently, the invading metastatic cell must display the appropriate functions to effectively colonize the new site.

Generating a Viable Niche

A novel mechanism for metastatic initiation has recently been proposed, which involves mobilization of hematopoietic progenitors from the bone marrow via circulation and into target sites for metastatic colonization in

response to hormonal factors emitted by primary tumors (Kaplan et al., 2005). Molecular characterization of these recruited hematopoietic cells identified them as expressing VEGFR1, CD133, CD34, and c-kit. These cells homed to and preconditioned sites of metastasis prior to dissemination of tumor cells from the primary site. Targeted inhibition of VEGFR1-expressing progenitors using neutralizing antibodies suggested that this preconditioning was necessary for metastatic progression. Furthermore, a subcutaneously inoculated lung carcinoma that induced these bone marrow-derived progenitors to congregate only in the lungs also metastasized only to that site, whereas a melanoma that recruited these progenitors to multiple organ sites exhibited a widespread metastatic tropism. Thus, cellular preconditioning by bone-marrow cells seems to contribute to organ-specific metastatic behavior.

What are the mechanisms of hematopoietic progenitor recruitment, and what does metastatic preconditioning entail? Although answers are pending, mice expressing a kinase-dead VEGFR1 have a decreased susceptibility to experimental lung metastases deriving from an implanted primary tumor (Hiratsuka et al., 2002). In this study VEGFR1 signaling was required for the premetastatic induction of MMP9 expression in endothelial cells and macrophages of the lungs by distant primary tumors (Hiratsuka et al., 2002). This premetastatic induction of MMP9 in the lung was postulated to condition the lung microenvironment to be more compliant for the invasion of metastatic cancer cells upon their arrival from circulation. Thus, VEGFR1 ligands and the downstream signals that they propagate may be required for the tumor-dependent recruitment of macrophage-like bone-marrow progenitors and/or for their premetastatic conditioning of target organ sites. Although several open questions still exist, this pathway exemplifies an important noncell autonomous aspect of metastasis and highlights the diversity of mechanisms involved.

In a State of Dormancy

The vast majority of tumor cells that have undergone extravasation still are not able to effectively colonize the new site. For several types of carcinomas, solitary tumor cells can be detected in the bone marrow years before the development of overt metastasis (Braun et al., 2005). The existence of such minimal residual disease (MRD) represents a predictive factor for disease recurrence and overall survival and is similar to lymph-node metastasis as an indicator of systemic disease (Pantel and Brakenhoff, 2004). Regardless, most of these cells will fail to convert into macrometastases.

What are the rate-limiting factors that leave tumor cells in a state of dormancy? Are these dormant cells the same ones that later give rise to overt metastases? What are the genetic and epigenetic dynamics of solitary disseminated cells? To answer some of these questions, experimental models of metastasis have been exploited. At least in some situations, an inability to induce angiogenesis at a secondary site appears to limit the outgrowth of micrometastases (Chambers et al., 2002). In this context, the acquisition of an angiogenic stimulus that is effective in the relevant secondary microenvironment could break a dormant state that is imposed by limited access to blood vessels. In other cases, however, disseminated tumor cells remain dormant in microenvironments where angiogenesis does not appear to be rate limiting. For reasons that are unclear, these solitary disseminated tumor cells are viable yet unable to re-enter the cell cycle. Perhaps due to their quiescence, adjuvant chemotherapy does not eradicate these solitary cells in breast-cancer patients (Pantel and Brakenhoff, 2004). Nonetheless, when similar dormant cells were extracted from liver tissue in a mouse model of breast cancer and reimplanted into the mammary fat pad, they were capable of forming tumors (Chambers et al., 2002). Dormant cells extracted from the bone marrow of cancer patients also possess a proliferative potential when plated in vitro, but to various degrees (Solakoglu et al., 2002). This heterogeneity in ex vivo proliferative potential is clinically significant: patients harboring MRD cancer cells that were more efficiently able to re-enter the cell cycle had a poorer survival than patients with MRD consisting of more quiescent cells (Solakoglu et al., 2002). Collectively, these findings suggest that disseminated tumor cells might enter a state of cell-cycle arrest, possibly induced by incompatibilities between the cancer cells and the secondary soil(s). Recall that, to a wandering cancer cell, no soil is really friendly but only tolerant at best.

Recent genomewide analyses of single cells are bringing the field closer to understanding the dynamics of MRD as it progresses into full-blown metastasis. The application of these technologies to disseminated cells from patients in defined stages of cancer progression has implicated dissemination as an early event in a cancer's history—after the onset of genomic instability but before the rampant imbalances evident during and after telomeric crisis (Pantel and Brakenhoff, 2004). The transition from MRD to overt metastasis in human breast, prostate, and gastrointestinal carcinomas has been proposed to coincide with the selective expansion of rare clones with distinct profiles of cytogenic abnormalities (Klein et al., 2002). Being able to dissect the genetic and epigenetic dynamics underlying the transition from dormancy to metastasis will be instrumental in advancing this area of study that, although technically difficult, is central to understanding metastasis.

Becoming Organ Specific

To escape dormancy or to colonize a new organ outright, disseminated tumor cells must have the capacity to productively interact with the new microenvironment in order to extract growth and survival advantages. Because different organ microenvironments may impose distinct requirements on cancer cells for full-blown colonization, the mediators of organ-specific metastasis may not

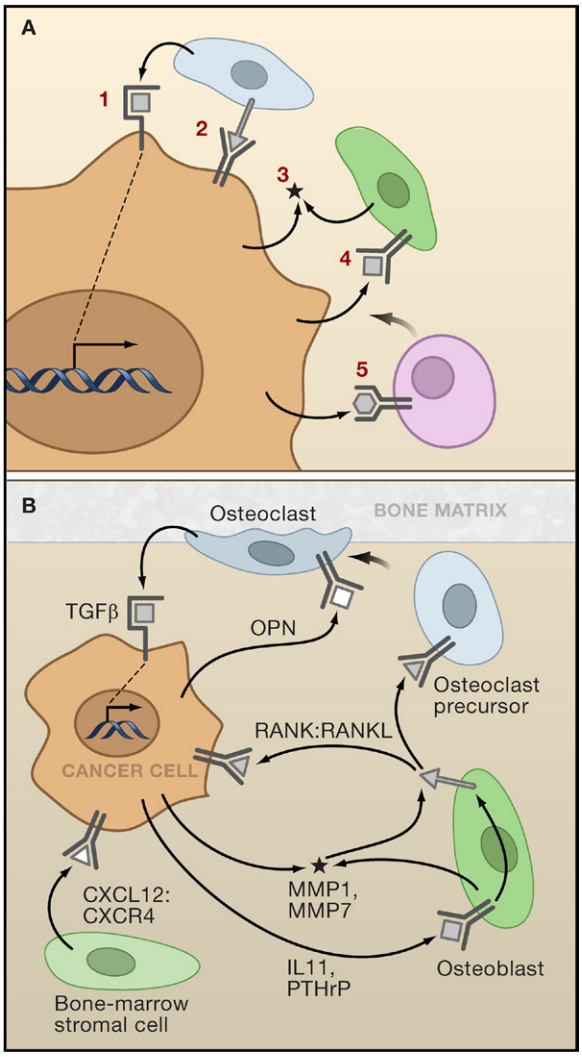

Figure 5. Site-Specific Mediators of Metastasis

(A) Conceptual categories of functions associated with site-specific metastasis genes. Factors in the microenvironment may directly act on malignant tumor cells (1) to modulate a cell-intrinsic property (such as self renewal) or to regulate transcription of other metastasis mediators. Tumor cells may express homing receptors (2) that recognize ligands present in the stroma of the metastatic microenvironment. Metastatic cells may release extracellular modifying effectors (3), such as proteases, to remodel the surrounding milieu to accommodate cellular invasion. Growth factors may be secreted by tumor cells to act on stromal cells in the local microenvironment (4) or at a distance (5). Associated stromal cells in the metastatic microenvironment may actively contribute to several of these mechanisms, as depicted in the diagram.

(B) A partial listing of known cellular and molecular players involved in osteolytic bone metastasis. The release of TGFβ from dissolved bone matrix drives further expression of some metastasis mediators, establishing what is commonly referred to as the "vicious cycle of bone metastasis."

always be the same (Figure 4). Mechanistic dissection of secondary organ colonization in model systems has begun to identify sets of mediators that may be necessary to complete this late stage of metastatic progression (Figure 5).

Colonizing Bone

The best characterized example of reciprocal cellular and molecular adaptations that occur between cancer cells and their stroma during site-specific metastatic progression comes from the analysis of tumors that metastasize to bone. A frequent site of distant metastasis, the skeletal system is composed of diverse cell types. When metastatic breast cancer invades bone, it frequently presents clinically with painful fractures due to induced hyperactivation of bone-resorbing osteoclasts (Mundy, 2002). Advanced prostate cancer, to the contrary, predominantly involves the stimulation of bone-depositing osteoblasts—thus resulting in a net increase in bone density and eventual bone-marrow displacement (Logothetis and Lin, 2005). What enables metastatic cells to colonize the unique bone microenvironment, and what determines whether the lesion will be primarily osteolytic or osteoblastic? Years of careful modeling and mechanistic dissection of breast- and prostate-cancer bone metastasis have shed light on these questions.

Bone homeostasis is preserved through a balance between osteoblasts and osteoclasts. A highly conserved molecular circuitry maintains this balance under normal conditions and after acute injury. Regulatory signals can come from afar, such as calcitonin and parathyroid hormone, or they can originate locally within the bone to directly modulate the differentiation and activity of bone-remodeling cells (Harada and Rodan, 2003). Genetic studies have linked Wnt and BMP signals to the differentiation of osteoblasts from bone-marrow mesenchymal precursors, perhaps in part through the induction of Runx2, a "master" transcription factor in the osteoblast lineage (Harada and Rodan, 2003). Osteoclasts, derived from the monocytic branch of hematopoiesis, can be differentiated and stimulated through the expression of cytokines, including M-CSF, RANKL, IL-6, IL-8, and IL-11 (Boyle et al., 2003). The source for many of these factors, especially RANKL, is in fact the bone-forming osteoblasts—thus establishing a cellular and molecular cycle for local bone homeostasis (Mundy, 2002).

Cancer cells that successfully metastasize to bone invariably hijack these conserved physiological mechanisms. Whereas prostate cancer cells frequently secrete osteoblast-promoting factors, including BMPs, Wnt-family ligands, endothelin-1, and PDGF, osteolytic breast-cancer cells frequently inhibit these pathways by secreting soluble inhibitors while overexpressing osteoclast-inducing factors such as PTHrP (parathyroid hormone-related protein), IL-8, and IL-11 (Logothetis and Lin, 2005; Mundy, 2002). In addition, there is frequent disruption of the homeostatic RANK-RANKL loop between osteoclasts and osteoblasts. Metastatic prostate carcinomas can secrete high amounts of the RANKL inhibitor osteoprotegerin, thereby attenuating osteoclastic reactions during metastasis (Logothetis and Lin, 2005). Conversely, osteolytic cancer cells can secrete proteases that cleave RANKL into a more

active form, thereby activating the osteoclasts (Lynch et al., 2005). Moreover, because bone is a rich source of matrix-embedded growth factors such as IGF and TGF-β, these cytokines are released upon osteolysis and act upon the invading tumor cells to promote the induction of osteoclast-promoting factors (Kang et al., 2005; Kang et al., 2003; Mundy, 2002; Yin et al., 1999). This positive feedback loop, referred to as "the vicious cycle of osteolytic bone metastasis," may indeed be the driving force for the selection of osteolytic mechanisms during the development of bone metastases.

In a genomewide analysis of genes selected for during bone metastatic progression in a breast-cancer xenograft model, several microenvironmental regulators were identified that suggested diverse biological functions (Kang et al., 2003). These included the homing and survival chemokine receptor CXCR4, the extracellular modifiers osteopontin and CTGF (connective tissue growth factor), the matrix metalloprotease MMP1, and the osteoclastogenic cytokine IL-11 (Figure 5). Interestingly, this bone-metastasis signature was manifest in rare pre-existing clones within the unselected malignant cell population, which itself expressed genetic markers indicative of general metastatic potential (Kang et al., 2003). Indeed, the parental cell line was a heterogeneous cellular outgrowth derived from the pleura of a patient with disseminated breast cancer, which was comprised of cells exhibiting diverse site-specific metastatic potential when xenografted into immunocompromised mice (Minn et al., 2005b). This heterogeneity in metastatic potential—a hallmark of cells from advanced breast-cancer patients—could not be explained by even subtle differences in expression of a previously described "poor prognosis signature" (Minn et al., 2005b). These observations are in line with the seed-and-soil hypothesis in that metastatic colonization of a secondary organ requires multifunctional site-specific metastasis programs above and beyond those genes that may promote general metastatic potential.

Colonizing the Lungs

Pulmonary involvement is relatively common among patients with metastatic disease of many different types of primary tumors, including breast cancer, gastrointestinal tumors, melanoma, sarcoma, and kidney cancer. A partial explanation for this high prevalence may be that our entire cardiac output circulates through the lung-capillary network. Most commonly, lung metastatic lesions initiate at the level of small pulmonary arterioles, where they must either burst through or otherwise breach both the tight endothelial junctions of lung blood vessels and the underlying basement membrane. Once in the lung parenchyma, metastatic cells must survive and grow in this unique microenvironment, which contains highly organized extracellular matrix and specialized cell types for the purpose of respiration.

Many mouse models of cancer have been used for the study of lung metastasis. However, the clinical relevance of these models is not always apparent, especially when large numbers of cancer cells are inoculated directly into the venous circulation to force the seeding of the lungs. For this reason, it is important to validate the physiological relevance of mediators of metastasis identified using orthotopic models, as well as through the analysis of clinical specimens of primary tumors and metastases.

In pediatric sarcomas, the cytoskeletal anchoring protein ezrin appears to be facilitate tumor-cell extravasation into the lung parenchyma, and its expression correlates negatively with overall survival in osteosarcoma patients (Khanna et al., 2004). Expression analysis of clinical samples of primary and lung metastatic hepatocellular carcinoma (HCC) identified osteopontin as a candidate mediator that was validated through inhibitor studies in experimental models of HCC metastasis (Ye et al., 2003). In breast cancer, the TGFβ and NF-κB pathways have been implicated in lung metastasis, although the downstream mediators remain largely unknown (Luo et al., 2004; Siegel et al., 2003). Recently, examination of genes selected in vivo during lung metastasis of breast-cancer cells identified a gene-expression signature that was highly enriched in mediators of pulmonary metastasis. This diverse set of genes encoded for secreted factors (including epiregulin, CXCL1, and SPARC), cell-surface receptors (e.g., VCAM1 and IL13Rα2), extracellular proteases (e.g., MMP1 and MMP2), and intracellular effectors (e.g., Id1 and COX2), which cooperated to promote lung metastasis. Significantly, these genes were further validated in a cohort of primary breast tumors, where expression of these genes correlated with lung metastatic relapse in the corresponding patients. Intriguingly, the lung metastasis genes identified in this study were largely distinct from genes previously associated with metastasis to bone through in vivo selection from the same parental breast-cancer cell line (Minn et al., 2005a). This observation may reflect the different functions necessary to colonize the biologically disparate bone and lung microenvironments.

Colonizing the Brain

Metastatic colonization of the brain, although less frequent than metastasis to other organs, bodes an alarmingly poor prognosis. Primary tumors that metastasize to the brain include lung cancer, breast cancer, melanoma, renal carcinoma, and colon cancer. Metastatic cells may either colonize the brain parenchyma or thrive along the leptomeninges, which represents the most aggressive form of this disease. Vascular access to the central nervous system is tightly regulated through a specialized structure termed the blood brain barrier (BBB). The BBB is composed of tightly adjoined endothelial cells that are further lined by basal lamina and astrocyte foot processes (Abbott et al., 2006). Physiologically, this barrier is so restrictive that even serum proteins are excluded unless they are actively shuttled across by intracellular transport. During brain metastasis, the BBB must be compromised in order to allow extravasation of cancer cells. Once in the brain parenchyma, interactions with glial cells may further influence the ability of a metastatic

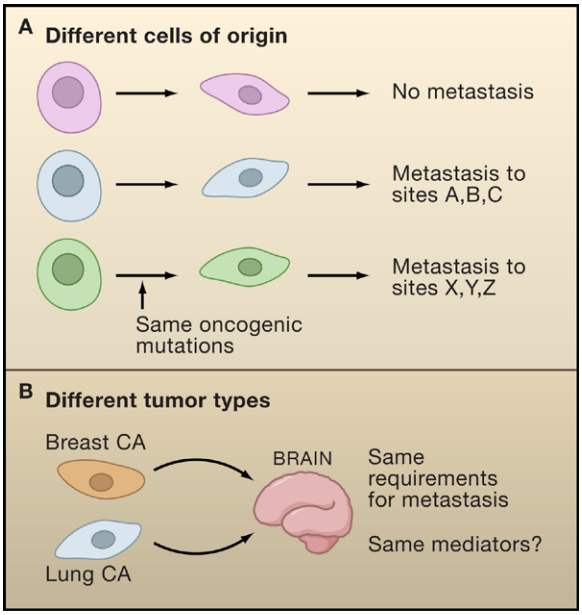

Figure 6. Impact of Cell of Origin on Metastatic Outcome

(A) Owing to differing predispositions encoded in cells with distinct developmental origins, the same oncogenic alterations may lead to diverse metastatic outcomes. In some cases, no metastatic behavior may be coupled to oncogenic transformation. In other instances, metastases might arise but may occur to different organs depending on which predisposing effectors are expressed.

(B) Two different tumor types may metastasize to the same secondary organ, such as breast and lung carcinomas metastasizing to the brain. It is currently unknown whether the developmental history of a cancer would result in different or common mediators of site-specific metastasis. Predisposing factors related to the cell of origin may engender different rate-limiting barriers during brain metastatic progression. Alternatively, different epigenetic accessibilities of the breast or lung cancer genomes may influence the likelihood of activating any particular genetic mediator of metastatic activity—ultimately resulting in unique genetic solutions to metastatic progression to the same secondary site.

cell to colonize it. Unfortunately, due to a dearth of reliable experimental models of brain metastasis, relatively little is known about mechanisms that enable brain colonization in mouse models, and even less so is known about what causes it in man.

Colonizing the Liver

The liver is a densely vascularized tissue that is supplied by both the portal and systemic circulatory systems. Advanced gastrointestinal malignancies tend to metastasize first to the liver, presumably due to direct perfusion through the portal vein. Other primary tumors can metastasize to the liver through the systemic vasculature, including breast cancer, uveal melanoma, and lung cancer. Unlike the restrictive blood vessels that perfuse the lungs and brain, hepatic sinusoids are discontinuously lined with endothelial cells, making them highly porous to blood nutrients and circulating cells. For this reason, extravasation may not be a major barrier to liver metastasis, as was observed in quantitative cell-tracking studies in an experimental mouse model (Chambers et al., 2002). Rather, invasion into the hepatic parenchyma and avoidance of cell death from resident immune cells may be rate-limiting steps during metastatic colonization of the liver. Indeed, expression of the hepsin protease was sufficient to enable liver metastasis of prostate-cancer cells in a transgenic mouse model without affecting proliferation of the primary tumor (Klezovitch et al., 2004). Additionally, periostin-mediated promotion of Akt signaling and cell survival in metastatic cells (Bao et al., 2004), as well as neutralization of proapoptotic TRAIL expression on natural killer (NK) cells resident in the liver (Takeda et al., 2001), are both mechanisms that facilitate hepatic metastasis in mouse models.

Seeding and Reseeding

Analysis of the aforementioned lung metastasis gene signature in a cohort of primary breast cancers using microarrays revealed a surprising result: some of the genes that promote metastasis were coexpressed within a subset of primary tumors (Minn et al., 2005a). Why would genes associated with and mediating aggressive lung metastasis from circulatory inoculation in mice be expressed in primary tumors to an extent that was detectable by microarray profiling? Orthotopic implantation into the mammary glands of mice revealed that cells expressing the lung metastasis genes generated primary tumors that grew more rapidly than the corresponding parental cell line (Minn et al., 2005a). Moreover, histological markers of cellular proliferation were unchanged, suggesting that this augmented growth was not simply a consequence of accelerated progression through the cell cycle.

One possible explanation for these phenomena may be that some of the genes that mediate metastasis have dual functions. That is, although these genes promote metastatic colonization, they may have been selected in the primary tumor for a function that provided a selectable growth advantage in the primary tumor microenvironment. Alternatively, it is possible that the properties conferred by metastasis-promoting genes—such as migratory capacity, proteolytic activity, self renewal, and extravasation—may, in and of themselves, allow for the progressive enrichment of cellular subpopulations exhibiting them in a primary tumor. For example, metastatically primed cells being shed at high rates from a primary tumor or from developing metastases may, at some finite frequency, travel back to the primary tumor. The intrinsic colonizing functions of such cells may allow them to constantly reseed the primary tumor—in effect metastasizing to their site of origin. It has been speculated on theoretical grounds that this would provide an effective means to achieve accelerated tumor growth (Norton and Massagué, 2006). If true, this phenomenon would partly explain why genes that promote metastasis are frequently expressed in primary tumors and why there is a consistent link between large tumor size, rapid growth rate, and metastatic behavior in a majority of cases of clinical cancer.

Impact of the Cell of Origin

Despite having gleaned some of the molecular underpinnings of organ specificity, we are still left pondering why different types and subtypes of cancer have varied propensities for metastasis, sometimes to specific secondary sites. Consideration of the original predisposition of the transformed cell of origin suggests several discrete possibilities that may explain these phenomena (Figure 6). Certain cell lineages may express molecules that bias the metastatic efficiency to different target organs. For example, normal mammary epithelial cells, as well as their cancerous counterparts, express RANK—the receptor for the previously discussed osteoclast differentiation factor RANKL. A recent study suggests that this receptor-ligand combination may predispose breast-cancer cells to bone colonization (Jones et al., 2006).

The developmental history of a cell may also predispose it to activate expression of specific metastasis-promoting mechanisms upon malignant transformation. This phenomenon has recently been demonstrated through the use of defined oncogenic elements to transform multiple normal human cell types into their cancerous counterparts, which are capable of generating tumors in immunocompromised mice (Gupta et al., 2005). Notably, whereas tumors derived from transformed fibroblasts and mammary epithelial cells were universally nonmetastatic, tumors generated from transformed melanocytes metastasized aggressively to secondary sites that are commonly affected in patients with malignant melanoma (Gupta et al., 2005). This difference was attributed to the selective expression of the transcription factor Slug in transformed melanocytes but not in transformed fibroblast or mammary epithelial cells. Slug is a developmental mediator of high migratory activity in the melanocytic precursors, neural crest cells.

How the developmental history of a cell influences the likelihood of expressing specific mediators of metastasis (such as Slug in melanoma) remains to be established. One possibility is that lineage-specific signaling and transcription-factor circuitries may alter the cellular response to the same oncogenic alterations. Alternatively, variegation in putative metastasis gene expression may be determined by developmentally imprinted transcriptional accessibility of the transformed cell genome. Epigenetic modifiers, including covalent histone modifications, DNA methylation, microRNA-mediated repression, or others, could be at play.

The impact of developmental predisposition on metastatic behavior is also important when transformation occurs at different stages within the same lineage. In breast cancer, for example, different cells of origin may partly explain the notable diversity in clinical manifestations of the disease. Global transcriptomic signatures have segregated breast cancer into a composite of four to five distinct subtypes, which may be malignant versions of different stages in the mammary-gland lineage. These signatures reveal patterns of overall metastatic likelihood and responsiveness to therapy (Perou et al., 2000). For example, tumors of the basal subtype share markers with the basal cell layer of normal mammary glands—where myoepithelial- and putative-tissue stem cells reside—and are noted for their aggressive metastatic proclivities. Alternatively, "luminal A" subtype tumors express the estrogen receptor and other markers of luminal epithelial cells in normal breast and are responsive to hormonally targeted therapies. However, this subclassification of breast cancer into subtypes does not typically inform about detailed metastatic behavior, such as the likelihood of relapse to a particular organ for a given patient with clinical breast cancer.

Finally, organism level predispositions for metastatic outcome may occur as polymorphisms or germline mutations. In principle, a mutation that alters the expression or activity of a mediator of metastasis may be phenotypically silent during the reproductive years of an individual and would consequently not be evolutionarily selected against. However, when one such individual develops cancer, the hyperactivity of the metastasis promoter may increase the likelihood that the tumor becomes metastatic. Support for this hypothesis has recently been provided using different inbred strains of mice that exhibit differing susceptibilities for lung metastasis in mammary tumors induced by the polyoma middle T oncogene (Park et al., 2005). Genetic mapping of the metastatic efficiency locus implicated a mutated hypermorphic allele of the GTPase-activating protein Sipa1 (Park et al., 2005). This discovery encourages the search for similar genetic associations in humans that might predispose individuals to an aggressive course of disease should they develop cancer.

Corollary and Outlook: An Evolving Framework for Metastasis

What began as a conceptualization of key steps and players involved in metastatic progression is progressively developing into a mechanistically rich depiction of how metastasis actually proceeds. Advanced modeling approaches, technological ingenuity, and an emphasis on clinical validation have all contributed to a rapid rate of recent progress. We can envision metastasis as one possible outcome from the somatic evolution of cancerous cells that have lost control over the integrity of their genome. The resulting cellular heterogeneity enables the selection for advantageous traits that allow malignant cells to overcome diverse environmental defenses, which normally preserve tissue structure and function. Primary tumors that continue to thrive in spite of these obstacles and exhibit genetic or phenotypic features indicative of stromal-cell co-option are often enriched in cells that are primed for the metastatic cascade. As these tumors go on to spew cells and soluble factors into the circulation, the entire body becomes an evolutionary playing field. Until the primary tumor is diagnosed and surgically removed, one might even imagine a period of dynamic interplay between cells in the primary mass

and those that have already undergone dissemination. Subsequent to surgical resection, however, the metastasis cascade falls beneath the radar. On occasion, rare clones from the collective pool of disseminated cancer cells may emerge that have adopted the requirements for growth in a secondary organ, one which they can now begin to colonize.

The stochastic nature of the metastatic cascade does not preclude the possibility of predicting the likelihood of its successful completion. By first understanding molecular mechanisms that facilitate metastasis—and subsequently observing the expression of these and other molecules in primary tumors and secondary sites of metastasis—the field has begun to assemble sets of genes whose expression or genomic status approximates the probability for specific metastatic outcomes. Importantly, we also realize that the pre-existing cellular state of a transformed cell—encoded genetically as well as epigenetically—can strongly predispose the resultant tumor to certain types of metastatic behaviors. Collectively, by considering these diverse aspects of metastatic progression, we have begun to understand not only how metastasis proceeds but also why it occurs in the first place.

As technologies advance and more pathways are implicated in metastatic progression, it becomes increasingly feasible to focus on molecular mediators that withstand rigorous tests for clinical relevance (that is, allowing researchers to focus on what does cause metastasis rather than on what can cause it). Because different tumor types may carry different predispositions, it is important to systematically establish the mechanisms utilized by each. It is entirely possible that two different tumor types metastasizing to the same secondary organ may select for entirely different sets of mediators due to disparate predisposing factors. For similar reasons, metastasis from tumors in a given organ to a particular secondary site may resort to different mediators in a mouse model and in man. Nonetheless, by relating the molecules utilized in each scenario to the necessary metastatic functions that they confer, we imagine that general concepts governing metastasis will emerge.

Genomic technologies will lead to the discovery of more biomarkers of metastatic likelihood, which should make it possible to develop reliable assays with clinical utility. Rigorous epidemiological practices should be adhered to as new genetic markers are validated. Recognizing the selective pressures in primary tumors that drive the emergence of metastatically primed cells might enable elucidation of signatures that are surrogate markers of specific prometastatic biological properties. Finally, "meta-signatures" must be designed that incorporate multiple prognostic gene sets into a singular assessment of the relevant biological properties of a malignancy as well as of its most likely course of clinical progression.

How can molecular insights into genes associated with metastasis be translated into new avenues for clinical metastasis therapy? Progress in this front is dependent on a separation of the mediators of metastasis from the markers of metastatic potential and a subsequent understanding of the necessary biological functions that are being selected for. A mechanistic appreciation for how these genes mediate cancer-cell growth and survival, as well as crosstalk with stromal-cell accomplices, will be necessary to identify stages of metastasis that might be susceptible to therapeutic intervention. The translation of these discoveries to patients should include a preliminary screening step to prospectively identify those cancer patients that might benefit most from any given targeted therapy approach. These are undoubtedly ambitious objectives, but the pace of recent progress suggests that the field is on track to making these goals a reality.

ACKNOWLEDGMENTS

We apologize to the many contributors to this field whose work we were unable to cite owing to space restrictions. We would like to thank Robert Benezra, Paula Bos, Anne Chiang, Piyush Gupta, Johanna Joyce, Yibin Kang, Andy Minn, Cristina Nadal, Don Nguyen, Larry Norton, David Padua, Howard Scher, and Harold Varmus for many insightful discussions. Our research on metastasis is supported by grants from the National Cancer Institute, the W.M. Keck Foundation, and the Kleberg Foundation.

REFERENCES

Abbott, N.J., Ronnback, L., and Hansson, E. (2006). Astrocyte-endothelial interactions at the blood-brain barrier. Nat. Rev. Neurosci. *7*, 41–53.

Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J., and Clarke, M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. USA *100*, 3983–3988.

Al-Mehdi, A.B., Tozawa, K., Fisher, A.B., Shientag, L., Lee, A., and Muschel, R.J. (2000). Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis. Nat. Med. *6*, 100–102.

Alitalo, K., Tammela, T., and Petrova, T.V. (2005). Lymphangiogenesis in development and human disease. Nature *438*, 946–953.

Allinen, M., Beroukhim, R., Cai, L., Brennan, C., Lahti-Domenici, J., Huang, H., Porter, D., Hu, M., Chin, L., Richardson, A., et al. (2004). Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell *6*, 17–32.

Bao, S., Ouyang, G., Bai, X., Huang, Z., Ma, C., Liu, M., Shao, R., Anderson, R.M., Rich, J.N., and Wang, X.F. (2004). Periostin potently promotes metastatic growth of colon cancer by augmenting cell survival via the Akt/PKB pathway. Cancer Cell *5*, 329–339.

Bartkova, J., Horejsi, Z., Koed, K., Kramer, A., Tort, F., Zieger, K., Guldberg, P., Sehested, M., Nesland, J.M., Lukas, C., et al. (2005). DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature *434*, 864–870.

Baylin, S.B., and Ohm, J.E. (2006). Epigenetic gene silencing in cancer - a mechanism for early oncogenic pathway addiction? Nat. Rev. Cancer *6*, 107–116.

Beachy, P.A., Karhadkar, S.S., and Berman, D.M. (2004). Tissue repair and stem cell renewal in carcinogenesis. Nature *432*, 324–331.

Bernards, R., and Weinberg, R.A. (2002). A progression puzzle. Nature *418*, 823.

Bhowmick, N.A., Chytil, A., Plieth, D., Gorska, A.E., Dumont, N., Shappell, S., Washington, M.K., Neilson, E.G., and Moses, H.L. (2004). TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science *303*, 848–851.

Bonnet, D., and Dick, J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. *3*, 730–737.

Boyle, W.J., Simonet, W.S., and Lacey, D.L. (2003). Osteoclast differentiation and activation. Nature *423*, 337–342.

Braun, S., Vogl, F.D., Naume, B., Janni, W., Osborne, M.P., Coombes, R.C., Schlimok, G., Diel, I.J., Gerber, B., Gebauer, G., et al. (2005). A pooled analysis of bone marrow micrometastasis in breast cancer. N. Engl. J. Med. *353*, 793–802.

Brodeur, G.M. (2003). Neuroblastoma: biological insights into a clinical enigma. Nat. Rev. Cancer *3*, 203–216.

Brown, D.M., and Ruoslahti, E. (2004). Metadherin, a cell surface protein in breast tumors that mediates lung metastasis. Cancer Cell *5*, 365–374.

Cavallaro, U., and Christofori, G. (2004). Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. Nat. Rev. Cancer *4*, 118–132.

Chambers, C.A., Kuhns, M.S., Egen, J.G., and Allison, J.P. (2001). CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. Annu. Rev. Immunol. *19*, 565–594.

Chambers, A.F., Groom, A.C., and MacDonald, I.C. (2002). Dissemination and growth of cancer cells in metastatic sites. Nat. Rev. Cancer *2*, 563–572.

Chang, H.Y., Sneddon, J.B., Alizadeh, A.A., Sood, R., West, R.B., Montgomery, K., Chi, J.T., van de Rijn, M., Botstein, D., and Brown, P.O. (2004). Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds. PLoS Biol. *2*, E7.

Chi, J.T., Wang, Z., Nuyten, D.S., Rodriguez, E.H., Schaner, M.E., Salim, A., Wang, Y., Kristensen, G.B., Helland, A., Borresen-Dale, A.L., et al. (2006). Gene expression programs in response to hypoxia: cell type specificity and prognostic significance in human cancers. PLoS Med. *3*, e47.

Clark, E.A., Golub, T.R., Lander, E.S., and Hynes, R.O. (2000). Genomic analysis of metastasis reveals an essential role for RhoC. Nature *406*, 532–535.

Condeelis, J., and Pollard, J.W. (2006). Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell *124*, 263–266.

Condeelis, J., and Segall, J.E. (2003). Intravital imaging of cell movement in tumours. Nat. Rev. Cancer *3*, 921–930.

Coussens, L.M., Fingleton, B., and Matrisian, L.M. (2002). Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science *295*, 2387–2392.

Criscuoli, M.L., Nguyen, M., and Eliceiri, B.P. (2005). Tumor metastasis but not tumor growth is dependent on Src-mediated vascular permeability. Blood *105*, 1508–1514.

Dannenberg, A.J., and Subbaramaiah, K. (2003). Targeting cyclooxygenase-2 in human neoplasia: rationale and promise. Cancer Cell *4*, 431–436.

de Visser, K.E., Eichten, A., and Coussens, L.M. (2006). Paradoxical roles of the immune system during cancer development. Nat. Rev. Cancer *6*, 24–37.

Douma, S., Van Laar, T., Zevenhoven, J., Meuwissen, R., Van Garderen, E., and Peeper, D.S. (2004). Suppression of anoikis and induction of metastasis by the neurotrophic receptor TrkB. Nature *430*, 1034–1039.

Egeblad, M., and Werb, Z. (2002). New functions for the matrix metalloproteinases in cancer progression. Nat. Rev. Cancer *2*, 161–174.

Erler, J.T., Bennewith, K.L., Nicolau, M., Dornhofer, N., Kong, C., Le, Q.T., Chi, J.T., Jeffrey, S.S., and Giaccia, A.J. (2006). Lysyl oxidase is essential for hypoxia-induced metastasis. Nature *440*, 1222–1226.

Feinberg, A.P., Ohlsson, R., and Henikoff, S. (2006). The epigenetic progenitor origin of human cancer. Nat. Rev. Genet. *7*, 21–33.

Felding-Habermann, B., O'Toole, T.E., Smith, J.W., Fransvea, E., Ruggeri, Z.M., Ginsberg, M.H., Hughes, P.E., Pampori, N., Shattil, S.J., Saven, A., and Mueller, B.M. (2001). Integrin activation controls metastasis in human breast cancer. Proc. Natl. Acad. Sci. USA *98*, 1853–1858.

Fidler, I.J. (2003). The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat. Rev. Cancer *3*, 453–458.

Friedl, P., and Wolf, K. (2003). Tumour-cell invasion and migration: diversity and escape mechanisms. Nat. Rev. Cancer *3*, 362–374.

Gorelik, L., and Flavell, R.A. (2002). Transforming growth factor-beta in T-cell biology. Nat. Rev. Immunol. *2*, 46–53.

Gorgoulis, V.G., Vassiliou, L.V., Karakaidos, P., Zacharatos, P., Kotsinas, A., Liloglou, T., Venere, M., Ditullio, R.A., Jr., Kastrinakis, N.G., Levy, B., et al. (2005). Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature *434*, 907–913.

Guo, W., and Giancotti, F.G. (2004). Integrin signalling during tumour progression. Nat. Rev. Mol. Cell Biol. *5*, 816–826.

Gupta, P.B., Kuperwasser, C., Brunet, J.P., Ramaswamy, S., Kuo, W.L., Gray, J.W., Naber, S.P., and Weinberg, R.A. (2005). The melanocyte differentiation program predisposes to metastasis after neoplastic transformation. Nat. Genet. *37*, 1047–1054.

Hanahan, D., and Folkman, J. (1996). Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell *86*, 353–364.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell *100*, 57–70.

Harada, S., and Rodan, G.A. (2003). Control of osteoblast function and regulation of bone mass. Nature *423*, 349–355.

Harris, A.L. (2002). Hypoxia–a key regulatory factor in tumour growth. Nat. Rev. Cancer *2*, 38–47.

Hernando, E., Nahle, Z., Juan, G., Diaz-Rodriguez, E., Alaminos, M., Hemann, M., Michel, L., Mittal, V., Gerald, W., Benezra, R., et al. (2004). Rb inactivation promotes genomic instability by uncoupling cell cycle progression from mitotic control. Nature *430*, 797–802.

Hill, R., Song, Y., Cardiff, R.D., and Van Dyke, T. (2005). Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. Cell *123*, 1001–1011.

Hiratsuka, S., Nakamura, K., Iwai, S., Murakami, M., Itoh, T., Kijima, H., Shipley, J.M., Senior, R.M., and Shibuya, M. (2002). MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. Cancer Cell *2*, 289–300.

Hu, M., Yao, J., Cai, L., Bachman, K.E., van den Brule, F., Velculescu, V., and Polyak, K. (2005). Distinct epigenetic changes in the stromal cells of breast cancers. Nat. Genet. *37*, 899–905.

Hussain, S.P., Hofseth, L.J., and Harris, C.C. (2003). Radical causes of cancer. Nat. Rev. Cancer *3*, 276–285.

Jones, D.H., Nakashima, T., Sanchez, O.H., Kozieradzki, I., Komarova, S.V., Sarosi, I., Morony, S., Rubin, E., Sarao, R., Hojilla, C.V., et al. (2006). Regulation of cancer cell migration and bone metastasis by RANKL. Nature *440*, 692–696.

Kalluri, R., and Zeisberg, M. (2006). Fibroblasts in cancer. Nat. Rev.

Cancer *6*, 392–401.

Kang, Y., Siegel, P.M., Shu, W., Drobnjak, M., Kakonen, S.M., Cordon-Cardo, C., Guise, T.A., and Massagué, J. (2003). A multigenic program mediating breast cancer metastasis to bone. Cancer Cell *3*, 537–549.

Kang, Y., He, W., Tulley, S., Gupta, G.P., Serganova, I., Chen, C.R., Manova-Todorova, K., Blasberg, R., Gerald, W.L., and Massagué, J. (2005). Breast cancer bone metastasis mediated by the Smad tumor suppressor pathway. Proc. Natl. Acad. Sci. USA *102*, 13909–13914.

Kaplan, R.N., Riba, R.D., Zacharoulis, S., Bramley, A.H., Vincent, L., Costa, C., MacDonald, D.D., Jin, D.K., Shido, K., Kerns, S.A., et al. (2005). VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature *438*, 820–827.

Karhadkar, S.S., Bova, G.S., Abdallah, N., Dhara, S., Gardner, D., Maitra, A., Isaacs, J.T., Berman, D.M., and Beachy, P.A. (2004). Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature *431*, 707–712.

Karin, M. (2006). Nuclear factor-kappaB in cancer development and progression. Nature *441*, 431–436.

Khanna, C., Wan, X., Bose, S., Cassaday, R., Olomu, O., Mendoza, A., Yeung, C., Gorlick, R., Hewitt, S.M., and Helman, L.J. (2004). The membrane-cytoskeleton linker ezrin is necessary for osteosarcoma metastasis. Nat. Med. *10*, 182–186.

Kim, M., Gans, J.D., Nogueira, C., Wang, A., Paik, J.H., Feng, B., Brennan, C., Hahn, W.C., Cordon-Cardo, C., Wagner, S.N., et al. (2006). Comparative oncogenomics identifies NEDD9 as a melanoma metastasis gene. Cell *125*, 1269–1281.

Klein, C.A., Seidl, S., Petat-Dutter, K., Offner, S., Geigl, J.B., Schmidt-Kittler, O., Wendler, N., Passlick, B., Huber, R.M., Schlimok, G., et al. (2002). Combined transcriptome and genome analysis of single micrometastatic cells. Nat. Biotechnol. *20*, 387–392.

Klezovitch, O., Chevillet, J., Mirosevich, J., Roberts, R.L., Matusik, R.J., and Vasioukhin, V. (2004). Hepsin promotes prostate cancer progression and metastasis. Cancer Cell *6*, 185–195.

Kurose, K., Gilley, K., Matsumoto, S., Watson, P.H., Zhou, X.P., and Eng, C. (2002). Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. Nat. Genet. *32*, 355–357.

Langowski, J.L., Zhang, X., Wu, L., Mattson, J.D., Chen, T., Smith, K., Basham, B., McClanahan, T., Kastelein, R.A., and Oft, M. (2006). IL-23 promotes tumour incidence and growth. Nature *442*, 461–465.

Liotta, L.A., and Kohn, E.C. (2001). The microenvironment of the tumour-host interface. Nature *411*, 375–379.

Logothetis, C.J., and Lin, S.H. (2005). Osteoblasts in prostate cancer metastasis to bone. Nat. Rev. Cancer *5*, 21–28.

Luo, J.L., Maeda, S., Hsu, L.C., Yagita, H., and Karin, M. (2004). Inhibition of NF-kappaB in cancer cells converts inflammation- induced tumor growth mediated by TNFalpha to TRAIL-mediated tumor regression. Cancer Cell *6*, 297–305.

Lynch, C.C., Hikosaka, A., Acuff, H.B., Martin, M.D., Kawai, N., Singh, R.K., Vargo-Gogola, T.C., Begtrup, J.L., Peterson, T.E., Fingleton, B., et al. (2005). MMP-7 promotes prostate cancer-induced osteolysis via the solubilization of RANKL. Cancer Cell *7*, 485–496.

Maser, R.S., and DePinho, R.A. (2002). Connecting chromosomes, crisis, and cancer. Science *297*, 565–569.

Mehlen, P., and Puisieux, A. (2006). Metastasis: a question of life or death. Nat. Rev. Cancer *6*, 449–458.

Minn, A.J., Gupta, G.P., Siegel, P.M., Bos, P.D., Shu, W., Giri, D.D., Viale, A., Olshen, A.B., Gerald, W.L., and Massagué, J. (2005a). Genes that mediate breast cancer metastasis to lung. Nature *436*, 518–524.

Minn, A.J., Kang, Y., Serganova, I., Gupta, G.P., Giri, D.D., Doubrovin, M., Ponomarev, V., Gerald, W.L., Blasberg, R., and Massagué, J. (2005b). Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. J. Clin. Invest. *115*, 44–55.

Muller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M.E., McClanahan, T., Murphy, E., Yuan, W., Wagner, S.N., et al. (2001). Involvement of chemokine receptors in breast cancer metastasis. Nature *410*, 50–56.

Mundy, G.R. (2002). Metastasis to bone: causes, consequences and therapeutic opportunities. Nat. Rev. Cancer *2*, 584–593.

Nash, G.F., Turner, L.F., Scully, M.F., and Kakkar, A.K. (2002). Platelets and cancer. Lancet Oncol. *3*, 425–430.

Norton, L., and Massagué, J. (2006). Is cancer a disease of self-seeding? Nat. Med. *12*, 875–878.

Orimo, A., Gupta, P.B., Sgroi, D.C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., Carey, V.J., Richardson, A.L., and Weinberg, R.A. (2005). Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell *121*, 335–348.

Overall, C.M., and Kleifeld, O. (2006). Tumour microenvironment - opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat. Rev. Cancer *6*, 227–239.

Paget, S. (1889). The distribution of secondary growths in cancer of the breast. Lancet *1*, 571–573.

Pantel, K., and Brakenhoff, R.H. (2004). Dissecting the metastatic cascade. Nat. Rev. Cancer *4*, 448–456.

Pardal, R., Clarke, M.F., and Morrison, S.J. (2003). Applying the principles of stem-cell biology to cancer. Nat. Rev. Cancer *3*, 895–902.

Park, Y.G., Zhao, X., Lesueur, F., Lowy, D.R., Lancaster, M., Pharoah, P., Qian, X., and Hunter, K.W. (2005). Sipa1 is a candidate for underlying the metastasis efficiency modifier locus Mtes1. Nat. Genet. *37*, 1055–1062.

Paszek, M.J., Zahir, N., Johnson, K.R., Lakins, J.N., Rozenberg, G.I., Gefen, A., Reinhart-King, C.A., Margulies, S.S., Dembo, M., Boettiger, D., et al. (2005). Tensional homeostasis and the malignant phenotype. Cancer Cell *8*, 241–254.

Pennacchietti, S., Michieli, P., Galluzzo, M., Mazzone, M., Giordano, S., and Comoglio, P.M. (2003). Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell *3*, 347–361.

Perou, C.M., Sorlie, T., Eisen, M.B., van de Rijn, M., Jeffrey, S.S., Rees, C.A., Pollack, J.R., Ross, D.T., Johnsen, H., Akslen, L.A., et al. (2000). Molecular portraits of human breast tumours. Nature *406*, 747–752.

Puc, J., Keniry, M., Li, H.S., Pandita, T.K., Choudhury, A.D., Memeo, L., Mansukhani, M., Murty, V.V., Gaciong, Z., Meek, S.E., et al. (2005). Lack of PTEN sequesters CHK1 and initiates genetic instability. Cancer Cell *7*, 193–204.

Radtke, F., and Clevers, H. (2005). Self-renewal and cancer of the gut: two sides of a coin. Science *307*, 1904–1909.

Ruiter, D.J., van Krieken, J.H., van Muijen, G.N., and de Waal, R.M. (2001). Tumour metastasis: is tissue an issue? Lancet Oncol. *2*, 109–112.

Semenza, G.L. (2003). Targeting HIF-1 for cancer therapy. Nat. Rev. Cancer *3*, 721–732.

Siegel, P.M., Shu, W., Cardiff, R.D., Muller, W.J., and Massagué, J. (2003). Transforming growth factor beta signaling impairs Neu-induced mammary tumorigenesis while promoting pulmonary metastasis. Proc. Natl. Acad. Sci. USA *100*, 8430–8435.

Singh, S.K., Hawkins, C., Clarke, I.D., Squire, J.A., Bayani, J., Hide, T., Henkelman, R.M., Cusimano, M.D., and Dirks, P.B. (2004). Identification of human brain tumour initiating cells. Nature *432*, 396–401.

Solakoglu, O., Maierhofer, C., Lahr, G., Breit, E., Scheunemann, P., Heumos, I., Pichlmeier, U., Schlimok, G., Oberneder, R., Kollermann, M.W., et al. (2002). Heterogeneous proliferative potential of occult metastatic cells in bone marrow of patients with solid epithelial tumors. Proc. Natl. Acad. Sci. USA *99*, 2246–2251.

Staller, P., Sulitkova, J., Lisztwan, J., Moch, H., Oakeley, E.J., and Krek, W. (2003). Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. Nature *425*, 307–311.

Stupack, D.G., Teitz, T., Potter, M.D., Mikolon, D., Houghton, P.J., Kidd, V.J., Lahti, J.M., and Cheresh, D.A. (2006). Potentiation of neuroblastoma metastasis by loss of caspase-8. Nature *439*, 95–99.

Takeda, K., Hayakawa, Y., Smyth, M.J., Kayagaki, N., Yamaguchi, N., Kakuta, S., Iwakura, Y., Yagita, H., and Okumura, K. (2001). Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. Nat. Med. *7*, 94–100.

Tarin, D., Price, J.E., Kettlewell, M.G., Souter, R.G., Vass, A.C., and Crossley, B. (1984). Mechanisms of human tumor metastasis studied in patients with peritoneovenous shunts. Cancer Res. *44*, 3584–3592.

Valk-Lingbeek, M.E., Bruggeman, S.W., and van Lohuizen, M. (2004). Stem cells and cancer; the polycomb connection. Cell *118*, 409–418.

Varambally, S., Dhanasekaran, S.M., Zhou, M., Barrette, T.R., Kumar-Sinha, C., Sanda, M.G., Ghosh, D., Pienta, K.J., Sewalt, R.G., Otte, A.P., et al. (2002). The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature *419*, 624–629.

Wang, H., Fu, W., Im, J.H., Zhou, Z., Santoro, S.A., Iyer, V., DiPersio, C.M., Yu, Q.C., Quaranta, V., Al-Mehdi, A., and Muschel, R.J. (2004). Tumor cell alpha3beta1 integrin and vascular laminin-5 mediate pulmonary arrest and metastasis. J. Cell Biol. *164*, 935–941.

Weigelt, B., Peterse, J.L., and van 't Veer, L.J. (2005). Breast cancer metastasis: markers and models. Nat. Rev. Cancer *5*, 591–602.

Weis, S.M., and Cheresh, D.A. (2005). Pathophysiological consequences of VEGF-induced vascular permeability. Nature *437*, 497–504.

Wicki, A., Lehembre, F., Wick, N., Hantusch, B., Kerjaschki, D., and Christofori, G. (2006). Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. Cancer Cell *9*, 261–272.

Yang, J., Mani, S.A., Donaher, J.L., Ramaswamy, S., Itzykson, R.A., Come, C., Savagner, P., Gitelman, I., Richardson, A., and Weinberg, R.A. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell *117*, 927–939.

Ye, Q.H., Qin, L.X., Forgues, M., He, P., Kim, J.W., Peng, A.C., Simon, R., Li, Y., Robles, A.I., Chen, Y., et al. (2003). Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. Nat. Med. *9*, 416–423.

Yilmaz, O.H., Valdez, R., Theisen, B.K., Guo, W., Ferguson, D.O., Wu, H., and Morrison, S.J. (2006). Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature *441*, 475–482.

Yin, J.J., Selander, K., Chirgwin, J.M., Dallas, M., Grubbs, B.G., Wieser, R., Massagué, J., Mundy, G.R., and Guise, T.A. (1999). TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. J. Clin. Invest. *103*, 197–206.

Zou, W. (2005). Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat. Rev. Cancer *5*, 263–274.