PERSPECTIVES

OPINION

The metastatic niche: adapting the foreign soil

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Abstract | The 'seed and soil' hypothesis for metastasis sets forth the concept that a conducive microenvironment, or niche, is required for disseminating tumour cells to engraft distant sites. This Opinion presents emerging data that support this concept and outlines the potential mechanism and temporal sequence by which changes occur in tissues distant from the primary tumour. To enable improvements in the prognosis of advanced malignancy, early interventions that target both the disseminating seed and the metastatic soil are likely to be required.

Steven Paget's 'seed and soil' hypothesis for metastasis was a pivotal milestone in the study of malignant disease. It introduced the concept that a receptive microenvironment was required for malignant cells to engraft distant tissues and form metastases[1,2]. Prior to this, the prevailing theory of the time was that the pattern of metastatic tumour dissemination was purely determined by mechanical factors that caused tumour cell emboli to lodge in the vasculature[3]. However, from his analysis of 735 cases of advanced breast cancer, Paget deduced that certain organs such as the liver appeared to be particularly receptive to metastases, and that this was not explicable by blood flow alone. He concluded that the 'soil' or local microenvironment of these organs must be more conducive for disseminating tumour cells to 'seed' than that of other organs such as the spleen. Forty years later, Paget's theory was challenged by James Ewing, who again proposed that metastasis was determined by the anatomy of the vascular and lymphatic channels that drain the primary tumour[4]. Ewing's view then prevailed until seminal studies by Isaiah Josh Fidler conclusively demonstrated that, although tumour cells reached the vasculature of all organs, metastases selectively developed in certain organs but not others[5,6]. Attention to the metastatic soil was revived, and a wealth of research ensued exploring the pathophysiology of the local tissue microenvironment, or 'niche', of cells of the primary tumour and that of tumour cells at metastatic sites.

The metastatic niche model

In ecological systems, the niche describes the interactive position of a species or population within a specific ecosystem. In the niche, the organism responds to the distribution of available resources and pressures of competitors, and in turn modulates the biological and physical components of its microenvironment by limiting access to other species and other actions. The place, status or activity for which a person is most suited can also be referred to as a niche. Similarly, in stem cell biology the niche describes the specialized microenvironment that supports stem cell maintenance and actively regulates cell function and proliferation[7–9]. A similar model has been suggested to delineate the interactions of malignant cells with their microenvironments at the primary tumour and at metastatic sites[10–12].

The soil of the primary tumour has been better characterized than that of metastatic sites. This microenvironment comprises supportive (non-malignant) stromal cells, soluble factors, vascular networks, nutrients and metabolic components, and the structural extracellular matrix (ECM) architecture[13–15]. A tumour-permissive immunological or inflammatory microenvironment is also required[16]. The metastatic niche model (FIG 1) suggests that a suitably conducive microenvironment (pre-metastatic niche) must evolve in order for tumour cells to be able to engraft (metastatic niche) and proliferate at secondary sites (micrometastatic to macrometastatic transition). These niches form as a result of tumour-secreted factors, and could either be newly induced or be adaptations of pre-existing physiological niches such as stem cell niches in haematopoietic organs. This hypothesis builds on Paget's seed and soil hypothesis by suggesting a temporal evolution for the development of the soil, and incorporates new data pertaining to the key cellular and molecular components of the metastatic microenvironment.

An alternative school of thought would argue that the intrinsic properties of the metastatic seed are more important determinants of metastasis than any contribution of the host microenvironment. Both this theory and the metastatic niche model are compatible with the generally accepted assumption that metastasis occurs in a stepwise fashion. Tumour cells detach from the primary tumour, invade and intravasate into the vasculature, and arrest in local capillaries in secondary organs where they extravasate, invade, survive and proliferate[17]. However, in contrast to other theories, the metastatic niche model presumes that the tumour cell does not solely dictate its own fate but that formation of a hospitable microenvironment is essential — not just permissive — to enable a disseminating tumour cell to spawn a secondary tumour growth.

The evidence for this model is primarily drawn from mouse models and largely focused on the lung as a target organ, although other organs such as liver, brain and bone have also been examined and patient studies have been conducted. Whether this model is widely applicable for solid tumour metastasis in general or whether it applies only to certain tumour types is not yet known. Furthermore, although there is substantial data describing the metastatic niche, the concept of a pre-metastatic niche is relatively novel and requires further study. The tissue parenchyma at target sites of metastasis is thought to adapt before the arrival of the first tumour cells as a result of systemic effects of factors

secreted by the primary tumour. However, defining the temporal sequence of events is dependent on the technical ability to detect single or small numbers of malignant cells in secondary organs. It is also possible that tumour cells condition their own metastatic microenvironments, thereby creating metastatic niches in a paracrine fashion.

Despite substantial advances in the treatment of localized malignancies, metastatic disease remains the primary cause of morbidity and mortality in cancer. The implication of the metastatic niche model is that, in order to improve the prognosis for patients with advanced malignancy, early therapeutic targeting of both the disseminating seed and the evolving metastatic soil is likely to be required. Moreover, therapies may need to be tailored to specific stages of the metastatic cascade.

The pre-metastatic niche

Mechanical forces of the vascular channels govern the initial delivery of cells from the primary tumour to distant tissues[18]. The anatomical route of vascular drainage from the primary tumour, vessel lumen diameter, blood flow and pressure, and the physical characteristics of the tumour cells all influence the locations in which the tumour cells are likely to arrest as they transit through the vasculature. Following adhesion and extravasation, survival and proliferation of tumour cells must occur efficiently for successful metastatic growth[19,20]. These processes require a receptive microenvironment at the destination site[17]. In recent years, evidence has emerged that growth factors secreted by the primary tumour prime certain tissues for tumour cell engraftment[19,21,22]. In response to these soluble factors, tumour-associated cells such as haematopoietic progenitor cells and macrophages cluster at 'pre-metastatic niches', creating an environment that is conducive for tumour cell adhesion and invasion[19,21] (FIG. 1). Indeed, in pre-metastatic organs, similar pathways may constitute homing signals for both tumour cells and tumour-associated cells such as haematopoietic cells[19,21]. Specific sites within organs that are primed in this fashion may be considered

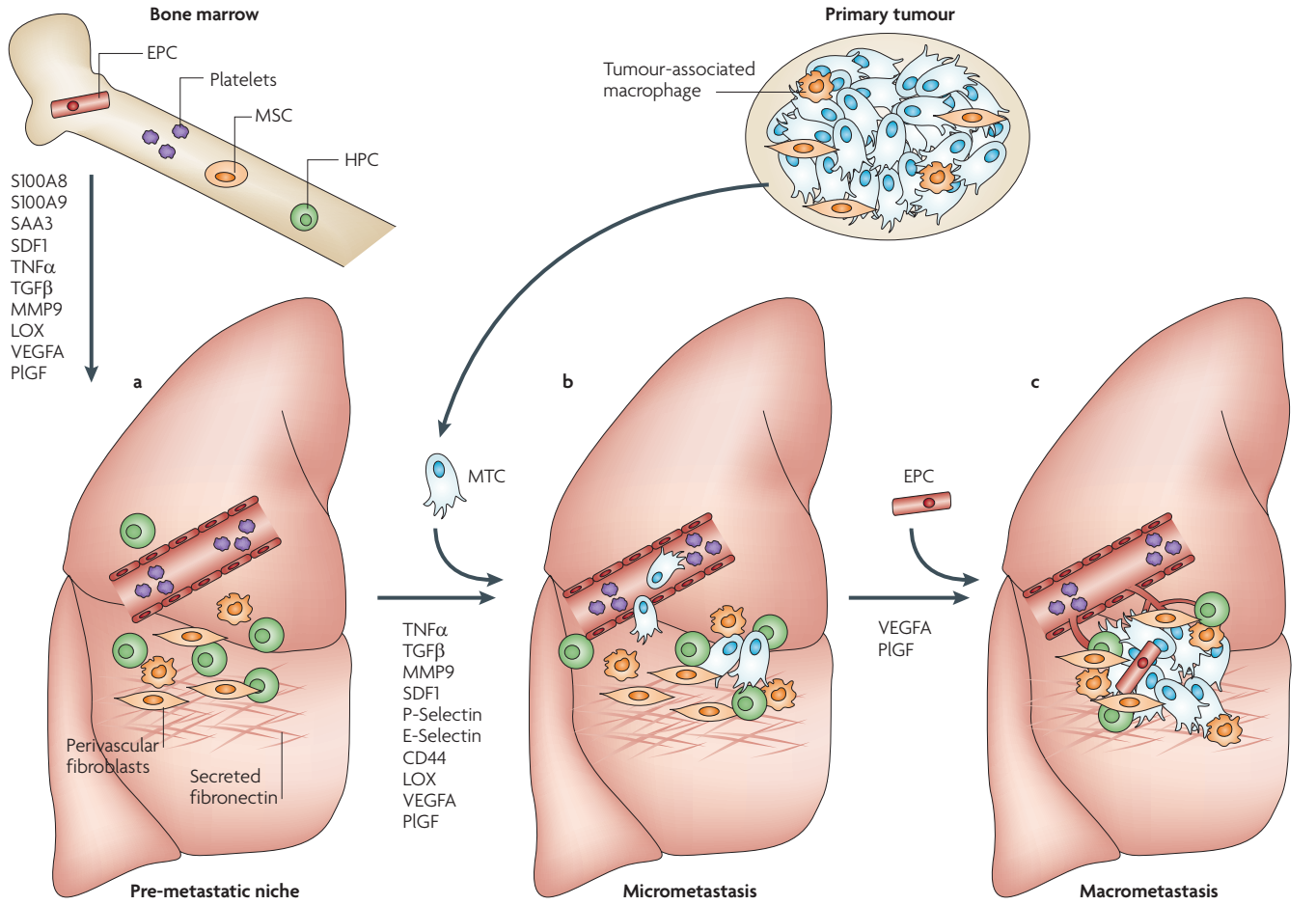

Figure 1 | **A model of the evolution of a metastatic niche.** This figure depicts the pre-metastatic, micrometastatic to macrometastatic transition. **a** | In response to growth factors secreted by the primary tumour, including vascular endothelial growth factor A (VEGFA)[19], placental growth factor (PlGF)[19] and transforming growth factor-β (TGFβ)[21], inflammatory S100 chemokines and serum amyloid A3 (SAA3)[21,22] are upregulated in pre-metastatic sites leading to clustering of bone marrow-derived haematopoietic progenitor cells (HPCs)[19]. Platelet-deployed stromal-derived growth factor 1 (SDF1) is also chemotactic for C-X-C chemokine receptor 4 (CXCR4)-positive HPCs and metastatic tumour cells (MTCs)[46]. HPCs secrete a variety of pre-metastatic factors including tumour necrosis factor-α (TNFα), matrix metalloproteinase 9 (MMP9) and TGFβ[19,36,37]. Activated fibroblasts, possibly derived from mesenchymal stem cells (MSCs), secrete fibronectin, an important adhesion protein in the niche, and lysyl oxidase (LOX) expression is increased, modifying the local extracellular matrix[55]. **b** | MTCs engraft the niche to populate micrometastases. The site-specific expression of adhesion integrins on activated endothelial cells such as P-selectin and E-selectin may enhance MTC adhesion and extravasation at these sites[97], and cell–cell interactions such as CD44 ligation in the metastatic niche may promote MTC survival and enable proliferation. **c** | Recruitment of endothelial progenitor cells (EPCs) to the early metastatic niche mediates the angiogenic switch and enables progression to macrometastases[19,100].

pre-metastatic niches. These evolve into metastatic niches following tumour cell engraftment. It appears that these niches preferentially develop at certain locations within an organ, such as around the terminal bronchioles and bronchiole veins in the lung[19], although this has not been definitively shown. In addition, differences between tumours in their pattern of metastatic dissemination appear to be a result of specific soluble factors secreted by the primary tumour. This was demonstrated in experiments in which mice bearing Lewis lung carcinomas (LLCs) received intraperitoneal injections of cell culture media that had been conditioned by B16 melanoma cells[19]. In this experiment, LLC metastasis was redirected to organs characteristic of melanoma but rarely seen with LLC metastasis, such as the spleen, intestine, kidney and oviduct[19].

Initiating the pre-metastatic niche. Cancer has long been associated with widespread mobilization of inflammatory cells in the blood and haematopoietic organs[23]. More recently, it was observed that bone marrow-derived haematopoietic cells that express vascular endothelial growth factor (Vegf) receptor 1 (VEGFR1) localize to pre-metastatic sites before the arrival of tumour cells[19]. These cells are of myeloid lineage and maintain their expression of immature surface markers including KIT and SCA1 within the tissue parenchyma, and are thought to be key components of the pre-metastatic niche[19] (BOX 1). The VEGFR1$^+$ cells also expressed the fibronectin receptor VLA4 (also known as integrin α4β1), and fibronectin expression was also noted to be increased at these sites[19]. The hypothesis that these localized accumulations of myeloid cells and stromal fibronectin were attractive docking sites for disseminating tumour cells set forth the concept that the induction of pre-metastatic niches within specific organs was a vital and permissive step for metastasis.

Mobilization of VEGFR1$^+$ myeloid cells from the bone marrow and their recruitment to pre-metastatic sites was initially thought to result mainly from the angiogenic cytokines VEGFA and placental growth factor (PlGF, a Vegf family member that binds specifically to VEGFR1) that are secreted by the primary tumour[19]. More recently, it was shown that inflammatory chemokines also recruit haematopoietic cells and tumour cells to pre-metastatic sites[21]. In examining the pre-metastatic lung in mice with syngeneic Lewis lung or B16 melanoma tumours implanted intradermally in the flank, Hiratsuka *et al.* reported that VEGFA, transforming growth factor-β (TGFβ) and tumour necrosis factor-α (TNFα) released by the primary tumour induced the expression of the inflammatory proteins S100A8 and S100A9 specifically within the parenchyma of the lung — the target site of metastasis — but not in other organs such as liver or kidney. This triggered infiltration by myeloid cells expressing the cell surface antigens integrin αM (also known as MAC1) or CD11b[21]. S100A8-stimulated lung was strongly chemoattractive for tumour cells in addition to MAC1$^+$ myeloid cells, and activation of the p38 MAPK signalling pathway was required for the recruitment of both cell types. Treatment with S100A8 and S100A9 antibodies inhibited the infiltration of MAC1$^+$ myeloid cells and resulted in a remarkable 80–90% reduction in tumour cell colonization of the lung, indicating that tumour cells and tumour-associated myeloid cells may respond to guidance signals through similar molecular mechanisms.

Selective upregulation of migration-stimulating factors in certain organs may contribute to the site-specificity of metastasis. In a recent extension of this work, serum amyloid A3 (SAA3) was shown to mediate S100A8- and S100A9-induced chemoattraction, acting through Toll-like receptor 4 (TLR4) on macrophages and tumour cells[22,24]. Moreover, the induction of the S100 chemokines and SAA3 occurred primarily in the lung, with minimal expression in liver or kidneys[10].

Simultaneously, cell–niche interactions occurring within the bone marrow enable mobilization of bone marrow-derived cells to the circulation in response to tumour-derived factors[25,26] (BOX 1). The cellular kinetics of bone marrow cells are regulated by a variety of cell types, including osteoblasts, osteoclasts, vascular endothelial and perivascular cells[27–30]. Whereas osteoblast-derived signals normally inhibit stem cell proliferation, it is thought that osteoclast and vascular signals promote proliferation and mobilization[31]. It is possible that, in the setting of metastatic progression, the balance alters in favour of stem cell mobilization from the bone marrow driven by endothelial cells and osteoclasts over osteoblast-mediated cell quiescence, although this has yet to be directly studied. The cell–microenvironment interactions occurring in the bone marrow are analogous to those between tumour cells and their stromal microenvironment at the primary tumour site and within pre-metastatic and metastatic niches. Indeed, it is possible that the bone marrow niches may be already well adapted to serve as metastatic niches, which may explain the higher survival rate of tumour cells within the bone marrow than in other organs in patients with malignancy[32]. The bone microenvironment appears to be particularly well suited as a metastatic site for many tumour types. This is attributed to the high expression of specific chemokines, such as stromal cell-derived factor 1 (SDF1), that promote tumour cell homing and engraftment, and the many nutrients that are released as a result of continuous bone remodeling[33].

Pre-metastatic niches: primed for tumour engraftment. At the pre-metastatic niche, newly recruited myeloid cells collaborate with other cell types including stromal cells and endothelial cells residing in the tissue parenchyma. Together, these cells provide a platform of chemokines, growth factors, matrix-degrading enzymes and adhesion molecules, thereby accelerating assembly of the metastatic lesion[21].

Box 1 | Postulated roles of BMDCs in tumorigenesis and metastasis

Several types of bone marrow-derived cells (BMDCs), derived from different origins, are involved in tumorigenesis and metastasis.

Haematopoietic cells

Haematopoietic progenitor cells are implicated as initiators of the pre-metastatic niche[19], and are involved in angiogenesis[25]. Mature monocyte and macrophage cells and neutrophils are also important in primary tumour and metastatic microenvironments[40,43]. These cells secrete factors, chemokines and matrix-degrading enzymes that modulate the local microenvironment and mediate the chemoattraction of other inflammatory cells to the pre-metastatic niche.

Endothelial cells

Endothelial progenitor cells are mobilized from the bone marrow during angiogenesis[25]. It has been suggested that recruitment of endothelial progenitor cells instigates the micrometastatic to macrometastatic switch[19,100].

Mesenchymal cells

Mesenchymal stem cells give rise to fibroblasts, which are important components of the tumour stroma[52,56]. They may also directly interact with tumour cells to enhance their metastatic phenotype[26].

Several inflammatory cytokines, including interleukin 1 (IL-1), IL-6, receptor activator of nuclear factor-κB ligand (RANKL, also known as TNFSF11) and TNFα are known to promote metastasis[16,34,35]. TNFα is produced by host myeloid cells and affects several steps in the metastatic process, including increased tumour cell proliferation, increased vascular permeability and the recruitment of other host cells[36]. Tumour-secreted factors directly induce myeloid cells to secrete tumour-promoting cytokines such as TNFα. A recent study exploring the molecular interaction between tumours and macrophages reported that the tumour-secreted matrix protein versican activated TLR2 on host macrophages leading to secretion of pro-metastatic inflammatory cytokines such as TNFα[36]. In this study, metastasis was severely abrogated in the absence of either TLR2 or TNFα. Few metastatic clusters were observed in the lungs of TLR2-deficicent mice inoculated with syngeneic LLC cells in a tail vein metastasis model[36].

Local tissue remodelling is essential to enable tumour cell invasion and metastatic outgrowth, and the expression of matrix metalloproteinases (MMPs) is also upregulated in the pre-metastatic niche[19,37]. MMPs are instrumental in degrading ECM components during inflammatory responses and tissue repair as well as in primary tumour growth[38,39]. MMP9 expression is specifically increased in endothelial cells and $MAC1^+$ and $VEGFR1^+$ myeloid cells in the pre-metastatic lung, in a VEGFA-dependent fashion[19,37]. MMP9 expression at pre-metastatic sites can serve both to facilitate tumour cell invasion and also to release growth factors and chemokines, including soluble KIT ligand, which further recruits bone marrow-derived progenitor cells and tumour cells that express the KIT receptor[19].

It is hypothesized that a major function of tumour-associated myeloid cells at the primary tumour site is to orchestrate other cells of the immune response to promote an immunosuppressive, anti-inflammatory phenotype and allow the tumour to escape immune detection[40]. For example, TGFβ production by $GR1^+CD11b^+$ myeloid cells directly interferes with $CD8^+$ cytotoxic T lymphocyte function and these cells also inhibit natural killer cells, B cells and the functional maturation of dendritic cells[40]. It is possible that myeloid cells recruited to pre-metastatic sites have a similar function: to create immune sanctuary sites in which malignant cells are able to survive and proliferate without detection. Expression of osteopontin by myeloid cells, a protein implicated in tumour cell adhesion and survival and in regulating MMP activity, also inhibits the host immune defence[41,42].

The molecular and functional phenotype of the myeloid cells that are recruited to pre-metastatic sites has yet to be fully characterized; the variation between laboratories in surface markers used to identify the cells compounds this challenge. In studies of the primary tumour, other groups have distinguished between $GR1^+MAC1^+$ immature myeloid cells (also known as myeloid-derived suppressor cells (MDSCs)) and terminally differentiated, $MAC1^+F4/80^+$ tumour-associated macrophages[43,44]. Both MAC1 and VEGFR1 are expressed on a wide variety of myeloid cells, including progenitor cells, and it is likely that both fully differentiated cells and immature cells are involved at the pre-metastatic and metastatic niche. There is some overlap between $VEGFR1^+$ and $CD11b^+$ cell subpopulations, although the precise lineage relationship is not known. It is thought that the $VEGFR1^+$ cells may be the first to be recruited and that these cells then produce factors that recruit or stimulate the proliferation of other myeloid cells[45].

In addition to myeloid cells, other cell types also play a part in establishing the pre-metastatic niche. For example, recruitment of $VEGFR1^+$ haematopoietic progenitor cells (which also express C-X-C chemokine receptor 4 (CXCR4)) to sites of neovascularization in ischaemic tissues and growing tumours is dependent on SDF1 released from platelet granules[46]. Although the role of platelets in the pre-metastatic niche has yet to be examined it is possible that they deliver chemokines and angiogenic regulatory factors here also[47,48]. Several tumour cell types also express CXCR4 and may therefore be influenced by platelet-derived SDF1 gradients, and platelet surface glycoprotein Ib-IX also appears to be important in mediating the colonization of the lung by metastatic melanoma cells in mouse models[49]. Other host cells resident at the pre-metastatic niche such as fibroblasts and endothelial cells may similarly express chemokines and adhesive proteins that attract circulating tumour cells to bind to these specific sites[50,51].

The transformation of local fibroblasts is pathologically important in the progression of cancer. Cancer-associated fibroblasts (CAFs) are perpetually activated, proliferating faster and depositing higher amounts of ECM components than resting fibroblasts in benign tissue[52]. CAFs have important roles both in the initiation of tumorigenesis and in malignant progression, facilitating proliferation, invasion and motility of malignant cells and constituting a source of MMPs for matrix degradation[19,53–56]. There is also evidence that fibroblasts are important in forming pre-metastatic niches. Activated fibroblasts have been shown to induce the stromal remodelling required for the development of liver metastasis in a murine melanoma model[57]. A proliferation of stellate cells, the fibroblasts that surround the liver sinusoids, was observed in association with early melanoma micrometastases. These cells were hyperactivated, secreting MMPs and chemotactic factors that fostered a conducive early metastatic microenvironment[57]. Subsequently, hypoxic induction of angiogenic growth factors (primarily VEGFA) in stellate cells recruited endothelial progenitors to the metastatic niche, facilitating the transition from micrometastases to angiogenic macrometastases[58].

A subpopulation of $CD45^+CD13^+$ mesenchymal cells referred to as fibrocytes has also been shown to contribute to the stromal changes in the pre-metastatic lung by upregulating MMP9 synthesis, which was functionally correlated with tumour engraftment[54]. However, whether these cells were locally recruited or bone marrow derived was not determined in this study. Intriguingly, in the setting of non-malignant kidney fibrosis, it has been reported that activated fibroblasts not only arise through epithelial–mesenchymal transition (EMT) and recruitment from the bone marrow, but also may emerge through endothelial–mesenchymal transition[59].

The ECM at the pre-metastatic niche. Alterations in tissue architecture are a hallmark of malignant disease[60]. As described above, myeloid cells and activated fibroblasts secrete factors such as MMPs that modulate the ECM. In addition, non-cellular factors such as local O_2 levels may also play a part. Tissue hypoxia has been associated with several aspects of malignant progression including metastasis[61]. The expression of lysyl oxidase (LOX), an enzyme that crosslinks collagens and elastins in the ECM, is upregulated in and secreted by hypoxic human tumour cells[62]. LOX secretion has been shown to substantially increase the invasive migration of a human breast cancer cell line both *in vitro* and *in vivo* in murine studies[63]. Recently it was suggested that secreted LOX may be important for the formation of pre-metastatic niches in target organs[53,55]. LOX secreted by hypoxic breast cancer cells accumulated at pre-metastatic sites, where it modified the ECM by crosslinking collagen fibrils to make it more receptive for myeloid cell infiltration[55].

Moreover, inhibition of LOX synthesis in human breast cancer cells reduced accumulation of $CD11b^+$ myeloid cells in the pre-metastatic organs of mice with orthotopic flank tumours and prevented metastasis[55].

Fibronectin, an ECM glycoprotein involved in numerous cellular processes including embryonic cell migration and vascular development[64], also appears to be an important component of the pre-metastatic niche. Focal expression of fibronectin has been observed around the terminal bronchioles and bronchiolar veins in the lung, common sites for metastatic niches[19,55]. Whether this fibronectin is derived from host stromal cells or from tumour cells is not yet clear. Although expression of fibronectin at pre-metastatic niches in the murine lung appeared to occur before the arrival of the first metastatic tumour cells[19], studies of human tumour cell lines in immunodeficient mice using antibodies specific to human fibronectin indicated that at least some of the fibronectin is tumour cell derived[55]. Both LOX expression and the myeloid cell clusters co-localized with fibronectin, suggesting that fibronectin may be crucial in initiating the assembly of other constituents of the pre-metastatic niche. It was not clarified whether the tumour cell-derived proteins LOX and fibronectin are deposited locally by disseminating cells transiting through the pre-metastatic or early metastatic lung or whether they are carried systemically from the primary tumour.

The mechanical properties of the ECM, such as tissue elasticity and matrix stiffness, have been shown to have a direct effect on tumorigenesis, especially in the mammary gland[65]. Whether these properties also have a role at metastatic sites, and at what stage in its evolution they come into play (pre-metastatic, micrometastatic or macrometastatic) has not yet been addressed.

Blood vessel integrity at the pre-metastatic niche. At the primary tumour site, disruption of vascular integrity enables trafficking of extracellular proteins and inflammatory cells[21], and is crucial for tumour cell invasion at metastatic sites[66–68]. It is possible that changes to existing local microvasculature occur before the arrival of tumour cells at sites of future metastasis, encouraging extravasation and clustering of tumour-associated myeloid cells, activated platelets and the first tumour cells. Many tumour-derived soluble factors have angiomodulatory effects, most notably VEGFA. The endothelium of organs is heterogeneous[69], and it is possible that vascular leakiness may not be a generalized phenomenon but could occur at specific sites — both organ specific and site specific within organs — perhaps influencing the formation of metastatic niches in these sites. Tissue-specific angiogenic factors have been identified, such as endocrine gland-derived Vegf[70,71]. Endocrine gland-derived Vegf is only biologically active in specific cellular and tissue contexts: it is a potent mitogen, and a pro-survival and migration factor only for endothelium of the adrenal cortex and gonadal tissue but not for aortic, umbilical or dermal microvasculature[71]. That tumours might secrete tissue-specific angiogenic molecules is appealing with respect to the formation of site-specific pre-metastatic niches; however, none have yet been identified in the context of metastasis. Alternatively, it is conceivable that tumour cells may produce tissue-specific inhibitors of angiogenesis and metastasis that prevent metastatic niche formation at certain sites, although this has not been shown.

One possible mechanism by which vascular permeability may be selectively modulated in certain organs is by the site-specific deployment of growth factors by circulating platelets depending on the presence of certain agonists. Recent studies showed that the activation of specific proteinase-activated receptors on the platelet surface may mediate selective deployment of pro-angiogenic versus anti-angiogenic growth factors[47,72]. Not only do platelets act as delivery vehicles for a myriad of angiogenic regulatory molecules, but the activated platelet surface also provides a platform of adhesive ligands such as P-selectin, to which circulating endothelial progenitor cells adhere in sites of angiogenesis[48].

Vascular endothelium cells may also influence the formation of the pre-metastatic and metastatic niches through differential expression of adhesion molecules at certain sites[73]. Endothelial expression of P-selectin and E-selectin is induced by inflammatory cytokines such as IL-1 and TNFα, promoting attachment of leukocytes to specific areas of endothelium, and these receptors have also been shown to mediate the attachment of cancer cells to activated endothelial cells. In one report, overexpression of E-selectin in multiple organs altered the organ distribution of metastasis in a transgenic mouse model[74]. However, the metastatic patterning did not correlate with the level of E-selectin expression in each organ, suggesting that other factors such as flow dynamics and shear stress of the blood supply also influence tumour cell attachment.

Pre-metastatic lymphangiogenesis. In the majority of cancer types, malignant spread to local lymph nodes occurs before solid organ colonization. However, although the number of studies focusing on tumour angiogenesis has exploded over the past few decades, the importance of establishing lymph vessel supply in the context of solid organ metastasis remains relatively unexplored. Overexpression of the Vegf family member VEGFC, one of the most potent lymphangiogenic growth factors, has been correlated not only with accelerated lymph node metastasis but also with lung metastasis, despite having no effect on the rate of primary tumour growth in a murine model of chemically induced squamous skin cancer[75]. Moreover, the onset of lymphangiogenesis within sentinel lymph nodes was demonstrated before tumour cell infiltration[75,76]. These data suggest that the induction of lymphatic vascularization may be an important preparatory step for tumour metastasis[77]. Whether lymphangiogenesis is important in the earliest stages of pre-metastatic niche formation in solid organs is not yet known.

The metastatic niche

In the metastatic niche model described here, significant changes occur in the local parenchyma at destination sites of future metastases that encourage subsequent homing and engraftment of circulating tumour cells (FIG. 1). Tumour cells then extravasate into local tissues and lodge in the pre-metastatic niche, where they may seed micrometastases and eventually form metastatic outgrowths.

Metastasis is an early event. The dissemination of malignant cells from the primary tumour to secondary sites was traditionally considered to be a late-stage event in terms of tumour progression and acquisition of malignant traits. However, several lines of evidence indicate that the initiation of metastasis may begin earlier in tumorigenesis than was previously thought. Advanced immunocytochemical and molecular techniques able to detect even single tumour cells have demonstrated that tumour cells are frequently present circulating in the blood and bone marrow of cancer patients before clinical or histopathological metastasis[32]. Indeed, elegant studies using transgenic mice that conditionally express oncogenes in mammary epithelial cells demonstrated that even untransformed mammary cells may lodge at secondary sites, where they can assume malignant growth following oncogene activation even

in the absence of detectable metastatic progression at the primary tumour site[78]. This suggests a novel hypothesis in which premalignant cells may disseminate during the early stages of tumour progression, and that malignant transformation of these cells may occur in ectopic microenvironments such as the pre-metastatic lung. It is possible that these premalignant cells may in fact prime their own microenvironments, that is, form the metastatic niche *in situ*[79], collaborating with local stromal cells to recruit myeloid cells and initiate the formation of a metastatic niche. Alternatively, circulating cancer cells that do not have metastatic potential may prepare sites for engraftment by more invasive cell subtypes[80]. Nonetheless, certain signals directed by the primary tumour must cause them to home to specific sites over others.

Tumour cell engraftment. Tumour cells appear to preferentially localize to the clusters of myeloid cells, fibronectin, growth factors and matrix remodelling proteins that constitute the pre-metastatic niche[19,53,55]. However, the molecular components that mediate the initial engraftment of tumour cells at these sites have yet to be fully characterized. Of the millions of cancer cells that enter the circulation, few will successfully engraft, survive and proliferate at secondary sites[81,82]. It is thought that, during haematogenous dissemination, the initial localization and extravasation of cells at secondary sites occurs efficiently, whereas the initiation and persistence of growth is inefficient[17]. This phenomenon may be determined both by the receptiveness of the local microenvironment where the tumour cells have sown[83] and also by cell-intrinsic factors that may provide a survival advantage in specific environments. The work by Massagué and colleagues identifying distinct genetic signatures of tumour cell subpopulations that correlate with a propensity for metastasis to specific organs has been pivotal in understanding the dynamics of tumour cell dissemination[72,84], and these studies are likely to have a major role in diagnostics and individualization of clinical management in the near future. The majority of these genes encode proteins that influence the interaction of tumour cells with the microenvironment, emphasizing the importance of favourable interactions with the soil of target sites for successful metastasis to occur[72,85]. In addition, expression of the transcriptional inhibitor of differentiation (Id) genes *Id1* and *Id3*, previously shown to be expressed in bone marrow progenitor cells mobilized for angiogenesis[86], also appears to be pivotal for metastatic colonization of the lung by human breast tumour cells, by facilitating sustained cellular proliferation during the early stages of colonization[87].

Other groups have investigated metastasis suppressor genes, which when re-expressed in malignant cells prevent metastasis without affecting their growth at the primary tumour site[88]. These genes may alter the ability of the cells to respond to survival signals received from the local microenvironment and thereby determine whether a certain microenvironment is permissive or inhibitory for the establishment of metastases. For example, expression of breast cancer metastasis suppressor 1 (*BRMS1*) in human breast cancer cell lines was shown to selectively attenuate responses to the mitogenic factors epidermal growth factor and platelet-derived growth factor, preventing colonization of distant tissues despite having no effect on primary tumour growth or haematogenous seeding of secondary sites in a mouse model[89].

In order to found secondary tumour growth in a foreign organ, malignant cells require the capacity to migrate and self-renew, properties similar to those exhibited by physiological stem cells and proposed properties of cancer stem cells[11,50,90,91]. The implication of this is that cancer stem cells may be more likely to successfully engraft in pre-metastatic niches. Indeed, recent evidence indicates that the process of EMT during early cancer invasion induces stem cell-like properties in breast cancer cells[92]. Inducing EMT in non-tumorigenic mammary epithelial cells led to the expression of proposed cancer stem cell antigenic markers $CD44^{high}CD24^{low}$ and acquisition of self-renewal and differentiation capacities[92]. It has also been suggested that fusion of tumour cells with macrophages may confer a migratory phenotype[93–95]. This intriguing hypothesis suggests that hybrids formed between tumour cells and primary tumour-associated macrophages may follow the same homing signals as the bone marrow-derived myeloid precursors to engraft pre-metastatic niches.

Metastatic tumour outgrowth. Following extravasation and invasion at the secondary site, tumour cell survival and proliferation may be influenced by cell–cell and cell–matrix interactions in the metastatic niche. For a disseminated tumour cell to successfully spawn a metastatic lesion, it must evade the numerous cell death signals that are induced by loss of attachment to neighbouring cells (anoikis) and the ECM (amorphosis), survive in the circulation and then productively communicate with the stroma of the foreign site[96]. The hyaluronic acid receptor CD44 has been shown to be especially important in enabling tumour cells to evade apoptosis during micrometastasis formation[97]. In mice injected through the tail vein with syngeneic mammary carcinoma cells, although inhibition of the interaction between CD44-bearing tumour cells and the lung matrix did not interfere with initial adherence to pulmonary endothelium or penetration of the interstitial stroma, the vast majority of carcinoma cells underwent apoptosis and were unable to form micrometastases[97]. In addition to hyaluronic acid, other ligands for CD44 include fibronectin, collagen I, osteopontin and laminin. Therefore, it is likely that specific interactions between tumour cells and molecular components of the metastatic niche such as fibronectin may be important in the evasion of cell death within the foreign soil. The metastatic niche would also constitute a rich source of growth factors and cytokines, many of which (including VEGFA) may directly regulate tumour cell proliferation in addition to survival.

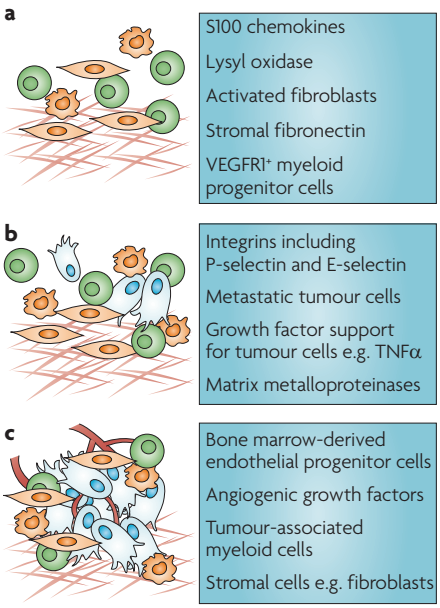

Figure 2 | **Stage-specific targeting of the metastatic microenvironment.** Cellular and molecular targets relevant to each stage of metastatic development (pre-metastatic (**a**), micrometastatic (**b**) and macrometastatic (**c**)) are suggested as ammunition for future anti-metastatic therapies. TNFα, tumour necrosis factor-α; VEGFR1, vascular endothelial growth factor receptor 1.

Micrometastatic to macrometastatic switch. The small proliferations of tumour cells at metastatic niches constitute micrometastases. Subsequently, the assembly of a functional vasculature is required to enable further cellular expansion and progression to macrometastases, a process for which activation of the angiogenic switch is required[98,99]. Recent studies exploring the cellular and molecular pathways that mediate the micrometastatic to macrometastatic switch identified bone marrow-derived endothelial progenitor cells (EPCs) as crucial regulators of this process[19,100]. The ID1 transcription factor, previously shown to be involved in primary tumour angiogenesis[25,86], appears to be crucial for mobilization of EPCs and their recruitment to micrometastases. Although short hairpin RNA inhibition of ID1 did not affect the initial colonization of the lung with tumour cells, angiogenesis and progression to macrometastases were prevented in the absence of EPC recruitment[100]. The functional contribution of the bone marrow-derived EPCs was particularly remarkable considering that they constituted less than 15% of the total endothelial cells in the metastatic vasculature[100]. In addition to EPCs, haematopoietic and mesenchymal cells aid macrometastatic progression. Tumour-associated macrophages potentiate the angiogenic stimulus by expression of VEGFA and angiopoietins, accelerate recruitment of other inflammatory cells and secrete proteases that further matrix remodelling[43].

The signals that initiate EPC recruitment and the angiogenic switch in the setting of dormant micrometastases and the molecular pathways underlying macrometastatic progression after EPC recruitment remain unclear. Further study is required to evaluate the role of the metastatic niche in tumour dormancy. Whether tumour cell dormancy results from dormant niches, or whether tumour cells regulate the activation state of the niches that they inhabit, is not known. In these scenarios, systemic factors such as tissue injury or ischaemia may be required to provide an angiogenic stimulus that reactivates the niche.

Implications for the clinic

The metastatic niche model carries several implications for the clinical management of advanced malignancy. First, immunohistological features of the pre-metastatic niche such as myeloid cell clusters, activated fibroblasts or stromal fibronectin may be used to identify a propensity to develop metastatic disease earlier than current prognostic techniques. In addition, examination of destination sites for metastasis may be used to distinguish patients who present with seemingly localized disease but have evidence of pre-metastatic niche formation and may therefore benefit from anti-metastatic therapies such as specific inhibitors of VEGFR1$^+$ myeloid cells, LOX or fibronectin.

Second, this model suggests that it may be beneficial for systemic therapies targeted to the metastatic microenvironment to be used early, perhaps even as an adjunct to the initial treatment of the primary tumour. If available, early interventions aimed at interfering with the formation of the pre-metastatic niche[101] may be particularly important in the treatment of malignancies that have a tendency to exhibit metastatic dormancy, such as breast carcinoma. Finally, there is the implication that treatments may need to be tailored to each stage of metastatic progression: pre-metastatic, micrometastatic and macrometastatic. Possible targets for future therapies are suggested in FIG. 2.

Limitations and unanswered questions

There are considerable limitations to the studies described above, and many questions remain unanswered. Examining truly pre-metastatic tissues in animal models is limited by the sensitivity and accuracy of tumour cell detection techniques. An even greater challenge lies in corroborating these data and confirming validity in the human setting, for which pre-metastatic and micrometastatic human tissue samples must be obtained.

The majority of studies of metastasis have focused on the lung as a metastatic organ, although other target sites such as liver and brain with established metastases have been examined. Furthermore, a wide variety of *in vivo* experimental models of metastasis are used in the studies described and each of these approaches carries specific limitations that need to be considered when interpreting the data. Although some studies have been corroborated in primary non-transplanted mouse models[19], the availability of these and of syngeneic mouse tumour cell lines is limited. Finally, owing to its highly complex cellular and molecular architecture, recapitulating the metastatic niche for *in vitro* studies is difficult.

Several outstanding issues require further clarification. For example, what are the implications of the metastatic niche model for metastatic tumour dormancy? What determines the specific localization of these niches within an organ? Are they newly initiated, or do pre-existing 'inducible niches' exist at certain sites? Following experimental intravenous injection of malignant cells, a minority will successfully engraft in certain sites, suggesting that there are pre-existing niches that do not need preparation by the primary tumour. If this is the case, are these related to physiological stem cell niches (BOX 2) and do differences in the genetic make-up of the host influence the number, capacity, location or efficiency of these niches? Is this model widely applicable or is there diversity between tumour types in

Box 2 | **Relevance to other physiological and pathological systems**

Interesting comparisons can be drawn between the cellular, molecular and functional phenotype of the metastatic niche and the physiological or pathological niches that occur in non-malignant conditions. For example, in reproductive physiology, the uterine wall is primed to accept the incoming, fertilized ovum, which for successful implantation must navigate the fallopian tubes and uterus in a migratory and invasive 'tumour-like' fashion. Implantation (invasion) of the developing blastocyst in the uterine wall requires extensive communication between the blastocyst and endometrium through interactions between surface integrins such as α5β1 and extracellular matrix proteins such as fibronectin[102,103]. In pathology, the anatomy of the focal inflammatory plaques seen in multiple sclerosis, atherosclerosis and rheumatoid arthritis also bear many similarities to that of the primary tumour microenvironment and the metastatic niche, with recruitment of cells and molecules known to be involved in metastasis, such as VLA4$^+$ monocytes, matrix metalloproteinases and osteopontin and microvasculature changes. Consideration of these analogous niches may suggest areas for study in metastasis research.

Physiological permissive microenvironments

- Germ cells and gametogenesis[104].
- Implantation of the blastocyst in the uterine endometrium[102,103].
- Maintenance of organ-specific stem cells in the adult, such as neuronal stem cells[105].

Pathological permissive microenvironments

- Multiple sclerosis[106].
- Atherosclerotic plaques[107,108].
- Rheumatoid arthritis[107,109].

their requirement for pre-metastatic conditioning for dissemination to occur?

We are just beginning to understand the complexities involved in the evolution of the metastatic niche, and many aspects discussed in this article remain speculative. Clearly, substantial progress is required before specific therapies that target the metastatic microenvironment are successful in the clinical arena. However, the preliminary insights highlighted here are integral steps towards identifying molecular and cellular targets for therapeutic development.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BRMS1
UniProtKB: http://www.uniprot.org
CD44 | CD24 | CXCR4 | E-selectin | fibronectin | *ID1* | *ID3* | KIT | IL-6 | MAC1 | PGF | P-selectin | RANKL | S100A8 | S100A9 | SDF1 | TLR2 | TLR4 | TNFα | VEGFA | VEGFC | VEGFR1 | VLA4

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OPINION

MicroRNAs — the micro steering wheel of tumour metastases

Milena S. Nicoloso, Riccardo Spizzo, Masayoshi Shimizu, Simona Rossi and George A. Calin

Abstract | Recently, microRNAs (miRNAs) have been discovered to have a role in metastasis. Here we describe how miRNAs are involved in advanced stages of tumour progression, stressing their roles as metastasis activators or suppressors, and discuss their possible use in the clinic as predictive markers and as therapeutic strategies for patients with metastases. Furthermore, we develop the concept that the same miRNAs could be involved both in the cancer stem cell phenotype and in the ability of specific cancer cells to produce metastases, thus representing a mechanistic link between the initial and the final steps of tumorigenesis.

The metastatic programme encompasses multiple sequential steps: cell motility, tissue invasion, intravasation, dissemination through the blood or lymph, extravasation and finally proliferation at a new site. However, the molecular pathways underlying each step are still obscure[1,2]. With the latest deciphering of roles for microRNAs (miRNAs) in the metastatic programme there are new hopes that this scenario will rapidly change. Since miRNAs were connected to cancer pathogenesis[3], accumulating data have pointed to a central regulatory role for miRNAs and other non-coding RNAs (ncRNAs; RNAs that do not have an open reading frame and do not encode protein) in the initiation and progression of most cancers analysed thus far. Recent studies show that miRNAs may be members of the still elusive class of cancer-predisposing genes and that other types of ncRNAs also participate in the genetic puzzle giving rise to the malignant phenotype (for reviews see REFS 4–7).

miRNAs and cancer

miRNAs were originally identified as small ncRNAs that control the timing of larval development in *Caenorhabditis elegans*[8]. miRNAs are short single-stranded RNA molecules, which serve as master regulators of gene expression (BOX 1). Their abnormal levels in tumours have important