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NON-THEMATIC REVIEW

The pre-metastatic niche: finding common ground

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Abstract It is rapidly becoming evident that the formation of tumor-promoting pre-metastatic niches in secondary organs adds a previously unrecognized degree of complexity to the challenge of curing metastatic disease. Primary tumor cells orchestrate pre-metastatic niche formation through secretion of a variety of cytokines and growth factors that promote mobilization and recruitment of bone marrow-derived cells to future metastatic sites. Hypoxia within the primary tumor, and secretion of specific microvesicles termed exosomes, are emerging as important processes and vehicles for tumor-derived factors to modulate pre-metastatic sites. It has also come to light that reduced immune surveillance is a novel mechanism through which primary tumors create favorable niches in secondary organs. This review provides an overview of our current understanding of underlying mechanisms of pre-metastatic niche formation and highlights the common links as well as discrepancies between independent studies. Furthermore, the possible clinical implications, links to metastatic persistence and dormancy, and novel approaches for treatment of metastatic disease through reversal of pre-metastatic niche formation are identified and explored.

Keywords Pre-metastatic niche · Hypoxia · Immunosuppression · Myeloid-derived suppressor cells · Exosomes · Tumor dormancy

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1 Metastasis: new thoughts

Metastasis remains the cause of over 90 % of cancer-related deaths from solid tumors [1]. The aggressive nature and widespread distribution of metastatic tumors limits the effectiveness of cancer therapeutics, and as such, a cure for metastatic disease remains elusive. The high mortality rate associated with metastatic disease emphasizes the need to move away from the current limiting paradigms regarding metastatic progression.

The process of metastasis is defined by distinct steps involving local invasion, intravasation into adjacent blood and lymphatic vessels, transit through circulation and evasion of host immune systems, extravasation into the parenchyma of distant organs, and colonization and formation of micrometastases, followed by proliferation and progression to macrometastases. This process is largely inefficient due to the many obstacles tumor cells must overcome to successfully metastasize and has until recently been regarded as a late event in tumorigenesis [2, 3]. Emerging evidence suggests that distinct forms of invasion

and metastasis may occur in different cancer types and that how, when, and where tumor cells metastasize needs to be explored in greater detail [1, 2, 4].

2 Learning from the past

Steven Paget's "seed and soil" hypothesis, proposed over a century ago, still forms the basis of our understanding of the metastatic process. In a study of 735 breast cancer autopsies, Paget noted that metastatic tumors were not randomly distributed in patients [5]. Instead, he proposed the "seed" (tumor cells) selectively colonized the "soil" of distant organs with an environment favorable for survival and proliferation [5]. It is now well established that specific organs are predisposed to metastases in certain cancers and that signaling between cytokines, chemokines, and their receptors regulates tumor cell homing to secondary organs [3]. An example is breast cancer, where tissues such as lungs, bone, liver, brain, and regional lymph nodes, which express high levels of stromal cell-derived factor-1 (SDF-1α/CXCL12), a ligand of the CXCR4 receptor expressed on breast tumor cells, are the most common sites of metastases [6, 7]. Yet, while chemokine signaling directs tumor cells to particular organs, the crosstalk between metastatic tumor cells, stromal and bone marrow-derived cell (BMDC) lineages once at the metastatic site is crucial in creating a supportive microenvironment. Creation of a metastatic microenvironment through the recruitment of BMDCs determines whether a disseminated tumor cell (DTC) survives and proliferates, becomes quiescent, or dies at metastatic sites [3]. The importance of the tumor microenvironment to primary tumor growth and progression is well established. The microenvironment at secondary sites of metastasis, while equally as important to allow metastatic tumor cell colonization and growth, is poorly understood in comparison. Recent evidence suggests the primary tumor itself is able to influence and alter the environment of secondary organs by promoting the formation of supportive metastatic microenvironments, termed pre-metastatic niches, prior to tumor cell dissemination.

3 The pre-metastatic niche: a new era in metastasis research

The components crucial to pre-metastatic niche formation include tumor-derived secreted factors (TDSFs) and BMDCs. TDSFs from the primary tumor promote the mobilization and recruitment of BMDCs that interact with the local stroma and extracellular matrix (ECM) at secondary organs, to help create microenvironments suitable for colonization by metastasizing tumor cells (Fig. 1).

The pre-metastatic niche was first described by Kaplan and colleagues in 2005 [8]. Tumor-derived VEGF and placental growth factor (PlGF) were demonstrated to promote the recruitment of $VEGFR1^+$ hematopoietic progenitor cells (HPCs) that formed distinct clusters of cells in secondary organs. Once at the secondary organ, clusters of $VEGFR1^+$ HPCs expressing the fibronectin receptor integrin VLA-4, interact with resident fibroblasts to stimulate fibronectin production and secrete MMP9 to create pre-metastatic niches for disseminating $CXCR4^+$ tumor cells. Subsequent research has identified various TDSFs and BMDCs important in pre-metastatic niche formation in different tumor models [9]. Although pre-metastatic niches are now widely accepted to be a true biological process promoting metastatic growth, speculation still exists as to whether their formation is necessary and required for metastases formation [10, 11]. While it is likely that pre-metastatic niches are not essential for metastases to form, various studies suggest that they greatly enhance the likelihood of metastatic progression [9]. This review will collate and explore the similarities and differences in the TDSFs and BMDC components implicated in pre-metastatic niche formation; highlight the roles of hypoxia, myeloid cells, and immunosuppression in regulating microenvironments at distant organs; discuss potential links to tumor dormancy; and investigate how this knowledge may help in the treatment of metastatic disease.

4 The primary tumor drives pre-metastatic niche formation: a role for hypoxia?

A variety of TDSFs including VEGF, PlGF, TNF-α, TGF-β, Lysyl oxidase (LOX), versican, and G-CSF have been shown to drive pre-metastatic niche formation in various tumor models (Table 1 and Fig. 1). While the role of individual TDSFs in promoting pre-metastatic niche formation is many and varied (Table 1), little has been done in the way of investigating the processes occurring at the primary tumor site to stimulate their initial production. Pre-metastatic niches may simply arise as a consequence of systemic disturbances caused by the presence of the primary tumor. The induction of angiogenesis, for example, is crucial to the development and growth of solid tumors and results in the production of many pro-angiogenic TDSFs including VEGF and PlGF from tumor cells and surrounding stromal cells such as bone marrow-derived macrophages [12], neutrophils [13], and mast cells [14]. Pre-metastatic niche formation could therefore simply be a bystander effect caused by the induction of angiogenesis at the primary tumor. Yet, while the influence of the primary tumor may be systemic, pre-metastatic niche formation does not appear to be, with niches generally observed in organs predisposed to metastases in certain cancer types as discussed later.

Defining the state or processes occurring in the primary tumor that result in the production of the pre-metastatic niche-

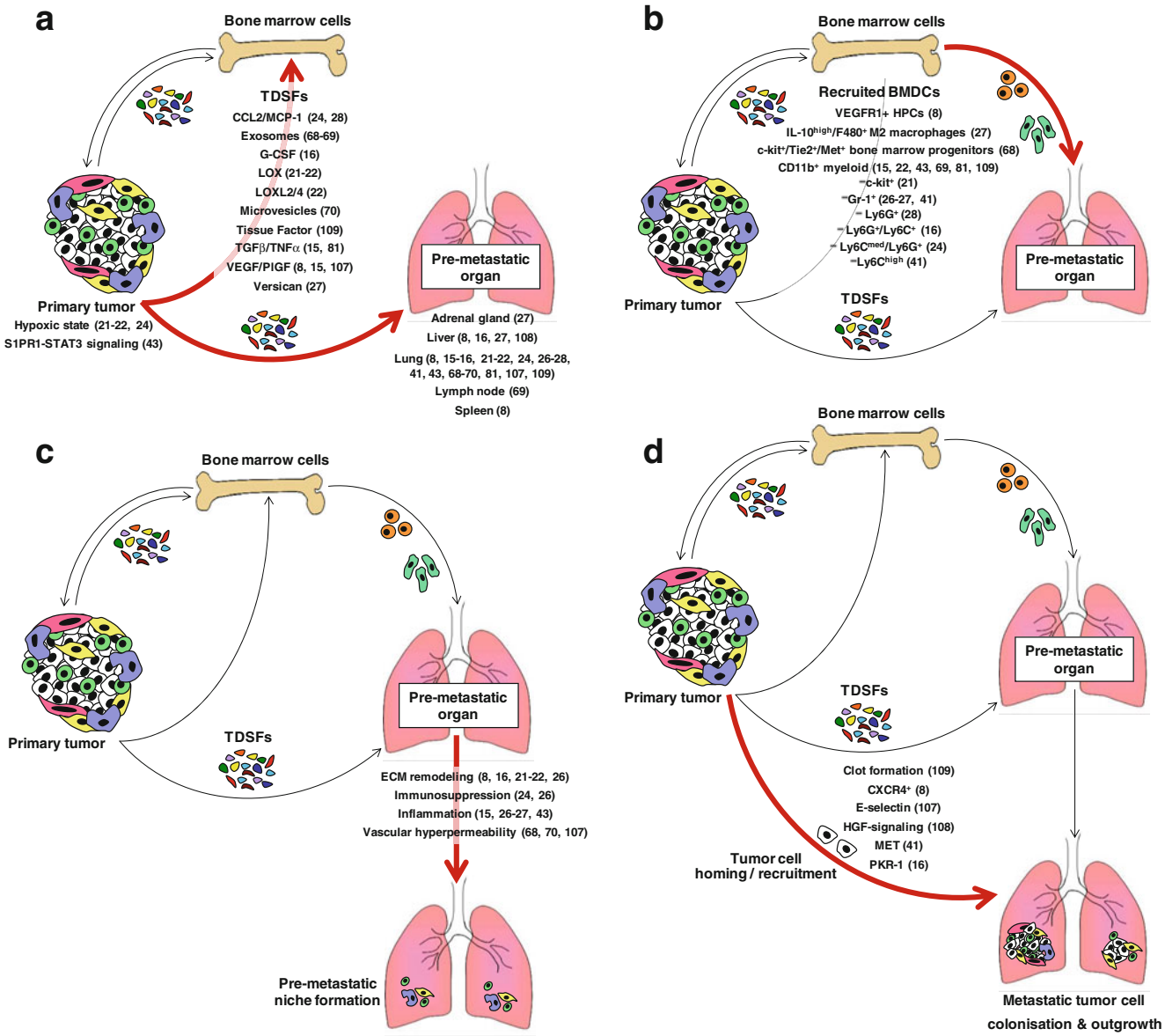

Fig. 1 Mechanisms of pre-metastatic niche formation. **a** The primary tumor is composed of both tumor cells and stromal cell lineages that create a pro-tumorigenic microenvironment at the primary site. As the primary tumor grows, it can become hypoxic, which is one process shown to be important in pre-metastatic niche formation. Tumor cells secrete a variety of TDSFs, including pro-angiogenic and hypoxia-dependent cytokines and growth factors, which influence various pre-metastatic organs including the lungs and liver. **b** Bone marrow-derived cells of hematopoietic and myeloid origin are mobilized from the bone marrow and recruited to pre-metastatic organs in response to TDSFs. A variety of myeloid cell lineages, defined by their expression of $CD11b^+$, and co-expression of other cell surface receptors including Gr-1, Ly6C, and/or Ly6G have been demonstrated to populate pre-metastatic organs. **c** The action of BMDCs at pre-metastatic organs helps to create niches by altering the microenvironment through processes including ECM remodeling, immunosuppression, inflammation, and vascular hyperpermeability. **d** The formation of pre-metastatic niches creates a supportive microenvironment allowing colonization and outgrowth of disseminated tumor cells that home or are recruited to secondary organs

promoting secreted factors is crucial to prevent niche formation, as many studies have demonstrated that silencing or neutralizing certain TDSFs can directly or indirectly decrease metastasis. Tumor-bearing mouse serum deprived of TNF-α, TGF-β, and VEGF-A by means of a neutralizing antibody for each protein, reduced the expression of proinflammatory proteins S100A8 and S100A9 in pre-metastatic lungs and reduced metastatic burden in a Lewis lung carcinoma (LLC) model [15]. Furthermore, administration of anti-G-CSF antibody to mice bearing 4T1, 66c14, or MMTV-PyMT tumors was shown to decrease pre-metastatic niche-promoting $Ly6G^+Ly6C^+$ myeloid cells in the peripheral blood and lungs, and thus decrease metastatic burden [16].

One process known to be associated with tumor progression, and more recently, pre-metastatic niche formation, is hypoxia. Hypoxia is a reduction in tissue oxygen tension

Table 1 Current published mechanisms of pre-metastatic niche formation

Reference	TDSFs	BMDCs	Mechanism of PMN formation	Anti- or pro-metastatic PMN (and PM organ)	Metastasis model	Animal models	Correlated with human disease
[8]	VEGF, PlGF	VEGFR1+ HPCs ($CD133^+$/$CD34^+$/c-kit^+)	TDSFs upregulate fibronectin in resident fibroblasts in PM organs to attract $VEGFR1^+$/$VLA\text{-}4^+$/$Id3^+$ HPCs that cluster and produce MMP-9, and SDF-1 which attracts $CXCR4^+$ tumor cells	Pro (lung, liver, spleen)	Primary tumor and experimental metastasis models	Lung (LLC^) and melanoma (B16^)	Yes
[15, 81]	VEGF, TGFβ and TNFα	$CD11b^+$/$Mac1^+$ myeloid cells	S100A8/S100A9 in PM lungs induces SAA3, which stimulates NF-κB signaling in a TLR4-dependent manner to generate an inflammatory state	Pro (lung)	Primary tumor and experimental metastasis models	Lung (LLC and 3LL^) and melanoma (B16^)	No
[21]	LOX secreted by hypoxic tumor cells	$CD11b^+$ myeloid and c-kit^+ myeloid progenitor cells	LOX accumulates with fibronectin and crosslinks collagen IV, allowing adhesion of MMP-2-producing $CD11b^+$ cells that create PMN through continued ECM remodeling and $CD11b^+$ cell recruitment	Pro (lung)	Orthotopic primary tumor and experimental metastasis models	Breast (MDA-MB-231† and 4T1^)	Yes
[69]	Exosomes supported by tumor-derived soluble fraction	$CD11b^+$ myeloid cells	CD44v expression on cancer-initiating cells is required to assemble a soluble matrix by which exosomes can activate leukocytes, stroma and endothelial cells in the PM organ	Pro (lung and lymph node)	Experimental metastasis model	ASML rat pancreatic adenocarcinoma	No
[27]	Versican	Myeloid cells including $CD11b^+$/Gr-1^+ and IL-10^{high}/$F4/80^+$ M2 macrophages	Versican secreted from primary tumor activates myeloid cells through TLR2 to produce TNFα to create inflammatory environment	Pro (lung, liver and adrenal gland)	Primary tumor and experimental metastasis models	Breast (4T1^) and lung (LLC^)	No
[16]	G-CSF	$Ly6G^+$/$Ly6C^+$ granulocytes	Bv8-expressing $Ly6G^+$/$Ly6C^+$ granulocytes induce MMP9, S100A8 and S100A9 and stimulate tumor cell migration mediated through PKR-1	Pro (lung and liver)	Orthotopic primary tumor and experimental metastasis models	Breast (4T1^, MDA-MB-231† and MMTV-PyMT^) lung (LLC^) and melanoma (B16F10^)	No
[26]	TGFβ (unpublished preliminary data)	$CD11b^+$/Gr-1^+ myeloid cells	$CD11b^+$/Gr-1^+ cells suppress immune response by reducing IFNγ, elevate inflammatory cytokines, and produce MMP9 to promote vascular remodeling and better tumor cell extravasation	Pro (lung)	Orthotopic primary tumor model	Breast (4T1^)	No
[70]	MVs shed from $CD105^+$ cancer stem cells	N/A	MVs shed from $CD105^+$ cancer stem cells contain pro-angiogenic mRNAs and miRNAs that enhance VEGFR1 on lung endothelial cells	Pro (lung)	Experimental metastasis models	Human renal cell carcinoma†	No
[28]	CCL2	$CD11b^+$/$Ly6G^+$ TENs from primary tumor site	Accumulation of cytotoxic TENs that kill metastatic tumor cells through apoptosis induced by contact-dependent secretion of H_2O_2	Anti (lung)	Orthotopic primary tumor & experimental metastasis models	Breast (4T1^) MMTV-Wnt1*, - PyMT*, - PyMT/cMyc*, & melanoma (B16^)	Yes
[107]	VEGF & PlGF	N/A	TDSFs including VEGF create regions of vascular hyper-permeability mediated by FAK activation on endothelial cells, that promotes homing of tumor cells through E-selectin upregulation	Pro (lung)	Orthotopic primary tumor & experimental metastasis models	Breast (EO771^) & lung (LLC^)	No
[108]	N/A	N/A	Increased TIMP-1 expression in PM organs activates Hif-1α-dependent HGF-signalling in tumor cells to promote invasion	Pro (liver)	Experimental metastasis model	L-CI.5s T-cell lymphoma	No
[22]				Pro (lung)	Orthotopic primary tumor models		Yes

Table 1 (continued)

Reference	TDSFs	BMDCs	Mechanism of PMN formation	Anti- or pro-metastatic PMN (and PM organ)	Metastasis model	Animal models	Correlated with human disease
	LOX, LOXL2, LOXL4 (Hif-dependent production)	$CD45^+/CD11b^+$ myeloid cells	Catalyzes collagen cross-linking in lung tissue to allow adhesion of disseminated tumor cells			Breast (MDA-MB-231† & 435†, MCF-7†)	
[43]	Production of LOX and fibronectin (and other TDSFs) upregulated by S1PR1-STAT3 signaling in tumor and myeloid cells	$CD11b^+$myeloid cells (also derived from primary tumor)	S1PR1 in primary tumor microenvironment activates STAT3 signaling in myeloid cells to promote their invasion, accumulation and survival. Persistent STAT3 activation in myeloid cells affects various components of PM organ microenvironment to promote PMN formation	Pro (lung)	Experimental metastasis model	Melanoma (B16F10^) & bladder (MB49^)	Yes
[41]	N/A	$CD11b^+/Gr\text{-}1^+$ myeloid cells ($CD11b^+/Ly6C^{high}$ fraction)	Secretion of versican from CD11b+/Gr-1+/Ly6Chigh fraction stimulates MET of metastatic tumor cells	Pro (lung)	Experimental metastasis models	Breast (MMTVPyMT^ & MDA-MB- 231†)	Yes
[109]	TF secreted from tumor cells	$CD11b^+$ moncytes/ macrophages	TF expressing tumor cells trigger clot formation, promoting localization of $CD11b^+$ monocytes, macrophages required for metastatic tumor cell survival	Pro (lung)	Subcutaneous primary tumor & experimental metastasis models	Melanoma (A7†; B16F10^)	No
[24]	Hypoxic-TDSFs including MCP- 1/ CCL2	$CD11b^+/Ly6C^{med}/Ly6G^+$ myeloid and $CD3^-/NK1.1^+$ NK cells	Decreased anti-tumor activity of NK cells in pre-metastatic lung allows disseminated tumor cell growth	Pro (lung)	Experimental metastasis model	Breast (PyMT & E0771^)	No
[68]	Exosomes expressing Met tyrosine kinase receptor	Exosome-programmed pro-vasculogenic $ckit^+/Tie2^+/Met^+$ bone marrow progenitor cells	Exosomes increase the metastatic behavior of primary tumors by 'educating' bone marrow progenitors via Met and induce vascular leakiness at pre-metastatic sites	Pro (lung)	Subcutaneous primary tumor model	Melanoma (B16F10^; SK-MES SK-Mel-28, SK-Mel- 202, SK-Mel-265 and SK-Mel-35†)	Yes

BMDC bone marrow-derived cell, *CCL2* chemokine (C–C motif) ligand 2, *ECM* extracellular matrix, *FAK* focal adhesion kinase, *G-CSF* granulocyte-colony stimulating factor, *HGF* hepatocyte growth factor, *Hif* hypoxia inducible factor, *HPC* hematopoietic progenitor cell, *IFN*γ interferon gamma, *LOX* lysyl oxidase, *LOXL* lysyl oxidase-like, *MCP-1* monocyte chemotactic protein-1, *MET* mesenchymal to epithelial transition, *Met* HGF receptor tyrosine kinase, *MMP9* matrix metalloproteinase 9, *MMTV* mouse mammary tumor virus, *MVs* microvesicles, *NK* natural killer, *PlGF* placental growth factor, *PKR-1* prokineticin receptor-1, *PM* pre-metastatic, *PMN* pre-metastatic niche, *PyMT* polyoma middle-T, *RAB* Ras-superfamily of GTPases, *S1PR1* sphingosine-1-phosphate receptor 1, *SDF-1* stromal cell-derived factor-1, *STAT3* signal transducer and activator of transcription 3, *TDSF* tumor-derived secreted factor, *TEN* tumor-entrained neutrophil, *TF* tissue-factor, *TGF*β transforming growth factor beta, *TIMP-1* tissue inhibitor of metalloproteinase-1, *TLR-2/4* toll-like receptor-2/4, *TNF*α tumor necrosis factor alpha, *VEGF* vascular endothelial growth factor, *VEGFR* vascular endothelial growth factor receptor. [a] *Transgenic mouse models; [b] ^ syngeneic transplantable mouse models; [c] † human cells in xenograft models

and occurs in all solid tumors larger than 1 cm^3 due to an inadequate blood supply resulting from the aberrant vasculature present in most solid tumors [17]. Cancer cells undergo genetic and adaptive changes to allow them to survive in hypoxic conditions, resulting in the promotion of an aggressive tumor phenotype clinically associated with metastasis and poor patient outcome. The hypoxia-inducible factors (HIFs) are the main downstream regulators of the hypoxic response-signaling pathway. Hif-1α is one isoform commonly associated with increased tumor growth, vascularization, and metastasis in various animal models and clinical studies [18]. Hif-1α overexpression correlates with increased patient mortality in early-stage lymph node negative-breast cancers [19] and predicts early relapse as shown in a retrospective study of 745 breast cancer patients [20]. In the absence of oxygen, HIF-1, a dimeric transcription factor formed by the oxygen-dependent Hif-1α and constitutively expressed Hif-1β subunits, binds to hypoxia-response elements in the nucleus, thereby activating the expression of numerous hypoxia-response genes [17].

Direct evidence for the role of primary tumor hypoxia in the promotion of pre-metastatic niche formation is demonstrated by the LOX family of proteins. HIF-dependent LOX and LOX-like (LOXL) proteins have been shown to remodel the ECM in pre-metastatic organs [21, 22]. LOX, secreted by hypoxic tumor cells, colocalizes with fibronectin in pre-metastatic sites and cross-links collagen IV in the basement membrane to promote the adhesion of MMP2-secreting $CD11b^+$ BMDCs [21]. β-Aminopropionitrile, an irreversible inhibitor of LOX, is only able to reduce metastasis in mice if administered prior to tumor cell injection [23], indicating a crucial role for LOX in the pre-metastatic phase. Targeting of LOX and LOXL proteins using two distinct Hif inhibitors, digoxin and acriflavine, can also inhibit lung metastases by inhibiting the cross-linking function of LOXL2 and LOXL4 in the pre-metastatic niche [22].

Importantly, the role of primary tumor hypoxia in promoting pre-metastatic niche formation is not just limited to the LOX and LOXL proteins. LOX proteins represent just a fraction of the total genes upregulated under hypoxia. Hypoxic-response genes drive many processes crucial to tumorigenesis including angiogenesis, glucose and iron metabolism, stress response, cell adhesion, proliferation, as well as drug resistance. Some of the most well-known hypoxic response target genes that promote metastatic progression include VEGF, LOX, LOXL2, LOXL4, TGF-β, MMP2, MMP9, CXCR4, and SDF-1 [18], all of which have indirectly or directly been linked to the pre-metastatic niche previously (Table 1). Recently, hypoxic tumor cells were demonstrated to be one of the main sources of pre-metastatic niche-promoting TDSFs [24]. A combination of factors secreted by hypoxic breast tumor cells, including those previously identified in pre-metastatic niche formation, and especially monocyte chemotactic protein-1 (MCP-1/CCL2), were able to stimulate the increased recruitment of BMDCs to pre-metastatic lungs [24]. Furthermore, mice treated with conditioned media from hypoxic breast tumor cells showed increased metastatic burden in multiple breast tumor murine models [24], indicating TDSFs from hypoxic tumor cells alone are capable of initiating pre-metastatic niches.

Although the process of hypoxic signaling in tumors results in the production of many pre-metastatic niche-promoting TDSFs, cell lines of various tumor origins express different hypoxic gene signatures. Two breast cancer cell lines, EO771 and PyMT-WT, analyzed for the production of various factors secreted in hypoxic-conditioned media, showed distinct hypoxic-TDSF signatures with minimal overlapping factors [24]. Furthermore, differences in TDSF production have been demonstrated in metastatic *versus* non-metastatic cell lines in breast cancer [16], and expression of LOX and LOXL proteins differs among breast cancer cells of varying metastatic capabilities under hypoxia [22]. Thus, TDSF signatures differ from cell line to cell line even amongst cancers of similar origin, depending on their metastatic capability and exposure to hypoxia. Therefore, developing an overall signature of pre-metastatic niche-promoting TDSFs will be difficult due to the heterogeneity amongst cancers and cancer cell lines, yet the metastatic capacity and hypoxic nature of a tumor appears to be a crucial determinant of pre-metastatic niche formation.

In experimental models, factors secreted from tumor cells have been identified as the pre-metastatic niche-initiating factors. However, in spontaneous cancer, both the tumor cells and the stromal components that make up the primary tumor microenvironment could release factors capable of changing the composition of pre-metastatic organs. The contribution of the tumor microenvironment to pre-metastatic niche formation, especially under hypoxic conditions, has not been closely studied or shown. Factors secreted by hypoxic endothelial and immune cells, fibroblasts, and other stromal components of the tumor microenvironment could potentially have a substantial impact on the development of pre-metastatic niches. Additionally, it is highly likely that both tumor and stromal cells in the metastatic tumor microenvironment contribute to the initiation of other metastatic events, although this remains to be properly investigated and raises further questions regarding the pre-metastatic niche and tertiary metastasis. For example, does pre-metastatic niche formation persist in a patient after surgical removal of the primary tumor, and will it be coordinated by the metastatic lesion? Does this give rise to pre-metastatic niche formation from already established metastatic lesions and would this change the organ specificity of pre-metastatic niches compared to the primary tumor alone? Clearly, we need to increase our understanding of many aspects of the metastatic process in order to rationally develop successful anti-metastatic drugs.

5 Myeloid cell diversity in the pre-metastatic niche is controlled by the local environment and tumor-derived factors

As discussed above, the disparity in TDSFs and their apparently different roles in pre-metastatic niche formation have generally been attributed to the different tumor models used. Indeed, mice pre-treated with conditioned media from melanoma tumor cells and then injected with LLC cells preferentially develop metastases in organs predisposed in melanoma and not lung cancer [8]. This suggests that the organotropism observed in different tumor types is largely determined and driven by the TDSFs secreted from the primary tumor. One method by which TDSFs promote this organotropism is through the mobilization of BMDCs to set up suitable environments in specific secondary organs. A common theme amongst different models of the pre-metastatic niche (Table 1) is the mobilization of myeloid cell lineages from the bone marrow and recruitment to specific pre-metastatic sites. The consequences of myeloid cell expansion in cancer are complex and varied. The interaction and communication between dispersed myeloid cells can lead to cancer cell proliferation, mutagenesis, angiogenesis, dissemination, and immune suppression through production of a variety of growth factors, proteases, pro-angiogenic molecules, and reactive oxygen and nitrogen species [25]. $CD11b^+$ myeloid cells are the BMDC lineage most commonly associated with pre-metastatic niche formation to date (Table 1 and Fig. 1). The pre-metastatic niche-promoting functions of these myeloid cells has been attributed to their integrin expression and production of various chemokines, growth factors, angiogenic factors, and inflammatory mediators in response to TDSFs (Table 1) [9].

A common myeloid progenitor derives from hematopoietic stem cells and can give rise to a variety of monocytic and granulocytic cell subtypes including macrophages, dendritic cells (DCs), neutrophils, and myeloid-derived suppressor cells (MDSCs) [25]. MDSCs, an immunosuppressive, immature myeloid cell lineage, have frequently been shown to accumulate in the pre-metastatic niche (Table 1) [15, 16, 24, 26–28]. MDSCs are associated with cancer progression in both animal models and humans [29–34], and accumulate in the bone marrow, blood, and spleen of tumor-bearing mice, as well as in the peripheral blood of cancer patients [30, 35]. Factors associated with MDSC expansion in cancer include known pre-metastatic niche TDSFs such as VEGF, G-CSF, S100A8 and S100A9, TGFβ, MMP9, and CCL2/MCP-1 (Table 2) [30, 36]. CCL2, S100A8, and S100A9 recruit MDSCs to the tumor stroma [37–39], while cytokines VEGF, GM-CSF, G-CSF, and M-CSF regulate myelopoiesis and inhibition of myeloid cell maturation [30, 36, 40].

The exact role of MDSCs in tumor progression depends on the subpopulation involved. MDSCs were originally defined by the co-expression of CD11b and Gr-1 antigens in tumor-bearing mice [36]. To date, two distinct populations of MDSCs have been characterized—monocytic MDSCs and granulocytic MDSCs (also known as polymorphonuclear MDSCs) [30, 36]. Antibodies against Gr-1 bind two distinct epitopes, Ly6G and Ly6C (encoded by different genes), distinguishing these $CD11b^+/Gr\text{-}1^+$ MDSC cells into two populations in mice, $CD11b^+/Ly6G^+/Ly6C^{low/med/+}$ granulocytic and $CD11b^+/Ly6G^-/Ly6C^{high/+}$ monocytic cells, with different functions in cancer, infection, and autoimmune diseases [30]. Human MDSCs are generally defined by the expression of the cell surface marker CD14 (among others), which distinguishes the monocytic ($CD14^+$) from granulocytic ($CD14^-/CD15^+$) subtypes [25, 36].

Differences in the nomenclature and cell surface markers used to define MDSC subpopulations have made collating the functions of a specific subtype difficult (Table 2). The diversity and inconsistency of MDSC subtype identification as a whole is reflected in our understanding of their role in the pre-metastatic niche. A $Ly6G^+/Ly6C^+$ granulocytic subset of $CD11^+/Gr\text{-}1^+$ myeloid cells, mobilized from the bone marrow by tumor-derived G-CSF, secrete MMP9, S100A8, and S100A9, as well as the chemoattractant protein Bv8, to enhance migration and homing of tumor cells as well as other $Ly6G^+/Ly6C^+$ cells in a positive feedback loop to form the pre-metastatic niche [16]. In contrast, $CD11b^+/Ly6G^+/Ly6C^{med}$ granulocytic myeloid cells mobilized from the bone marrow by hypoxic tumor cell-derived MCP-1 help to create an immunosuppressive pre-metastatic niche through suppression of natural killer (NK) cell cytotoxicity and maturity [24].

Secretion of versican from $CD11b^+/Gr\text{-}1^+/Ly6C^{high}$ myeloid cells pushes disseminating tumor cells from a mesenchymal to epithelial phenotype to promote metastatic colonization of the pre-metastatic niche [41]. Additionally, tumor-secreted versican activates $CD11b^+/Gr\text{-}1^+$ myeloid cells to produce TNFα, which in turn enhances cancer cell survival while recruiting leukocytes to create a proinflammatory pre-metastatic niche [27]. As well as promoting vascular remodeling and a proinflammatory environment through the secretion of factors such as MMP9, $CD11b^+/Gr\text{-}1^+$ myeloid cells can also suppress the immune response in the pre-metastatic niche through reduction of IFNγ [26].

In addition to influencing myeloid cells mobilized directly from the bone marrow to the pre-metastatic niche, TDSFs also influence the differentiation of myeloid cells in the primary tumor microenvironment, which in turn influences the myeloid cell subpopulations found in pre-metastatic organs. MDSCs that share similar phenotype and morphology have been shown to display functional differences dependent on their location at either the primary tumor site or peripheral lymphoid organs [42]. $CD11b^+/Ly6G^+$ myeloid cells that differentiate into tumor-entrained neutrophils (TENs) at the primary site actually prevent pre-metastatic

Table 2 Correlation between TDSFs implicated in pre-metastatic niche formation and their reported effects on MDSC populations in cancer

TDSFs	Effect on MDSC population or function	MDSC population	Murine tumor model	Human cancer association	PMN reference
CCL2/MCP-1	Expansion, mobilization & recruitment of MDSCs	$CD11b^+/Ly6C^+/Ly6G^-$ [44]	Lymphoma & thymoma	NA	[24, 28]
		$CD11b^+/Gr\text{-}1^{int/dull}/Ly\text{-}6C^{high}$ [94]	Lung & melanoma	NA	
		$CD11b^+/Gr\text{-}1^+$ [37]	Hepatocarcinoma & melanoma	Breast, gastric & ovarian	
G-CSF	Expansion, mobilization & recruitment of MDSCs	$CD11b^+/Gr\text{-}1^{high}/Ly\text{-}6C^{int}$ [94]	Lung & melanoma	NA	[16]
		$CD11b^+/Gr\text{-}1^+$ [95]	Lung, melanoma, myeloma & lymphoma	NA	
IFN-γ	Involved in MDSC-mediated suppression of lymphocytes	$CD11b^+/Ly6C^+/Ly6G^+$ [44]	Lymphoma & thymoma	NA	[26]
		$CD11b^+/Gr\text{-}1^+/IL\text{-}4R\alpha^+$ [54]	Colon carcinoma	NA	
		$CD11b^+/Gr\text{-}1^+$ [96]	Mammary adenocarcinoma	NA	
MMP9	Expansion of MDSCs and also secretion from MDSCs promotes angiogenesis	$CD11b^+/Gr\text{-}1^+$ [97]	Colorectal & lung carcinoma	NA	[8, 15, 16, 21, 26]
		$CD11b^+/Gr\text{-}1^+$ [98]	Mammary carcinoma	NA	
S100A8 & S100A9	Recruitment, accumulation & differentiation of MDSCs	$CD11b^{high}/Gr1^{high}/F4/80^-/CD80^+/IL4R^{+/-}$/Arginase [99]	Mammary carcinoma	NA	[15, 81]
		$CD11b^+/Gr\text{-}1^+$ [100]	Mammary carcinoma & lymphoma	NA	
TGFβ	Involved in MDSC-mediated suppression of lymphocytes	$CD11b^+/Gr\text{-}1^+$ [101]	Fibrosarcoma	NA	[15, 26, 27]
		$CD11b^+/Gr\text{-}1^+$ [38]	Mammary carcinoma	Breast	
Tumor-derived exosomes & microvesicles	Expansion and differentiation of MDSCs	$CD11b^+/Gr\text{-}1^+$ [77]	Melanoma and mammary carcinoma	NA	[68–70]
		$CD11b^+/Gr\text{-}1^+$ [102]	Mammary adenocarcinoma	NA	
		$CD14^+/HLA\text{-}DR^{-/low}$ [75]	NA	Melanoma and colorectal cancer	
VEGF	Expansion of MDSCs and promotion of angiogenesis by MDSCs	$CD11b^+/Gr\text{-}1^+$ [103]	Fibrosarcoma & endothelioma	NA	[8, 15]
		$CD11b^+/VEGFR1^+/CXCR4^+$ [104]	Renal cell carcinoma	Metastatic renal cell carcinoma	
		$CD11b^+/Gr\text{-}1^+$ [105]	Lung, melanoma, myeloma & lymphoma	NA	
		$CD14^+/HLA^-/DR^{-/low}$ [106]	NA	Colon, renal cell, cervical, pancreas, lung, mesothelioma, melanoma, fibrosarcoma & osteosarcoma	

CCL2, chemokine (C-C motif) ligand 2; G-CSF, granulocyte-colony stimulating factor; IFNγ, interferon gamma; MCP-1, monocyte chemotactic protein-1; MDSC, myeloid-derived suppressor cell; MMP9, matrix metalloproteinase 9; PMN, pre-metastatic niche; TDSF, tumor-derived soluble factor; TGFβ, transforming growth factor beta; VEGF, vascular endothelial growth factor

niche formation through targeted cytotoxicity of tumor cells in pre-metastatic organs [28]. In contrast, enhanced STAT3 signaling in $CD11b^+$ myeloid cells at the primary tumor site, mediated by TDSFs from STAT3-activated tumor cells, promotes the invasion, survival, and accumulation of these myeloid cells which drive pre-metastatic niche formation [43].

Thus, several factors appear to be crucial in determining the myeloid cell population accumulated in pre-metastatic niches. Firstly, TDSFs characteristic of different tumors undoubtedly play a role in the expansion and differentiation of particular subpopulations of myeloid cells. Secondly, the properties of the myeloid cells can be influenced by the characteristics of their local environment. Notably, whether myeloid cells are first mobilized from the bone marrow to the primary tumor microenvironment before migrating to pre-metastatic sites, or from the bone marrow to pre-metastatic niches directly, is an important determinant of their differentiation and function.

6 Immunosuppression as a mechanism of tumor promotion in the pre-metastatic niche

One function of these myeloid cells in the pre-metastatic niche that has not been thoroughly investigated is their potential to suppress important cellular mediators of the innate and adaptive immune responses. The monocytic and granulocytic MDSC subpopulations have distinct immunosuppressive functions. Granulocytic MDSCs are the prevalent population in the tissues and circulation of tumor-bearing mice, but are individually less immunosuppressive than monocytic MDSCs. Granulocytic MDSCs are closely

linked to $CD8^+$ T cell suppression through production of reactive oxygen species (ROS), whereas monocytic MDSCs suppress lymphocyte activation through production of enzymes ARG1, inducible nitric oxide synthase (iNOS), and ROS [34, 36, 44, 45]. There are four major mechanisms by which MDSCs are proposed to suppress immune cell function. These include depletion of amino acids required by lymphocytes, oxidative stress induced by production of ROS and reactive nitrogen species, interference with lymphocyte trafficking and viability, and lastly, activation and expansion of regulatory T (T_{reg}) cells [36].

Recently, $CD11b^+/Ly6C^{med}/Ly6G^+$ granulocytic myeloid cells were identified as the main myeloid cell constituent of the pre-metastatic niche, mobilized by hypoxic TDSFs [24]. These granulocytic myeloid cells are neutrophil precursors which include myelocytes and premyelocytes [36], whereas neutrophils are not immunosuppressive and are only released from the bone marrow when fully matured. Hypoxic TDSFs were also shown to specifically increase $CD3^-/NK1.1^+$ NK cells in pre-metastatic lungs. Although an increase in NK cells would normally be expected to reduce metastatic burden, NK cells in the pre-metastatic niche induced by hypoxic TDSFs showed reduced cytotoxic effector functions corresponding with a reduction in maturation [24], suggestive of pre-metastatic organs with reduced immune surveillance mediated by the presence of this suppressive population of granulocytic MDSCs. Increased $CD11b^{high}/Gr\text{-}1^{high}$ myeloid cell infiltration also causes decreased NK cell-mediated activity and enhanced metastatic burden in the lungs of pregnant mice [46], which demonstrated a similar gene signature to that of pre-metastatic lungs published previously [15, 46].

The exact method by which these MDSC populations suppress NK cells in general, not just in the pre-metastatic niche, is not well understood. MDSC-mediated NK cell suppression has been suggested to occur in a manner similar to contact-dependent T cell suppression. MDSC expansion in a cancer context reduces NK cell cytotoxicity by induction of anergy in hepatic NK cells *via* membrane-bound TGF-β1, resulting in decreased NKG2D expression and IFN-γ production [47], and through interaction with the NKp30 receptor on NK cells in human hepatocellular carcinoma [48]. Contact-independent mechanisms of NK cell suppression by MDSCs have also been reported. Granulocytic MDSCs ($CD11b^+/Ly6G^+/Ly6C^{lo}$) expanded in the context of *in vivo* adenoviral vector therapy are capable of NK cell suppression through ROS production, namely of H_2O_2 [49], while MDSCs from tumor-bearing hosts can reduce NK cell function through inhibition of IL-2-mediated activation and perforin production [50].

NK cells are now emerging as important mediators of immune responses at pre-metastatic sites, but the significance of other crucial mediators of the adaptive immune response remains to be seen. MDSCs are closely linked to suppression of T cells, especially $CD8^+$ T cells. MDSCs at the primary tumor site can suppress nearby T cells in a contact-independent manner [51–54] but, in peripheral lymphoid organs, have been reported to suppress $CD8^+$ T cells through direct cell–cell contact and antigen presentation [55, 56]. MDSCs affect the viability, proliferation, effector functions, and migration of T cells (reviewed in [36]). Hypoxia is also an important determinant in MDSC differentiation, which in turn dictates their ability to suppress T cells. The presence of Hif-1α in the primary tumor microenvironment is directly responsible for differentiation of $CD11b^+/Gr\text{-}1^+$ cells into antigen-nonspecific suppressors of T cell function *via* up-regulation of arginase and NO [42]. Therefore, another role for hypoxia in promoting pre-metastatic niche formation may be *via* the suppression of T cells (as well as NK cells), through the expansion and differentiation of MDSCs either at the primary tumor or pre-metastatic sites.

While T cells have not been directly linked to the pre-metastatic niche, there is some suggestion in previous studies to warrant further investigation into how this cell population may be affected by MDSCs at pre-metastatic sites. $CD3^+$ T cells have been identified in micrometastases of lungs containing clusters of $CD11b^+/Gr\text{-}1^+$ cells [27] and show increased proliferation when myeloid cell function is decreased by STAT3 ablation at pre-metastatic sites [43]. IFNγ is an important immunostimulatory and immunomodulatory cytokine secreted from many cells of the innate and adaptive immune responses including NK cells, NKT cells, as well as $CD4^+/CD25^+$ T_{reg} cells, $CD4^+$ and $CD8^+$ T cells. While a reduction in IFNγ secretion from macrophages has been reported by Yan and colleagues as a mechanism through which $CD11b^+/Gr\text{-}1^+$ MDSCs decrease the immune response in pre-metastatic sites [26], macrophages are not generally a major source of IFNγ, so it is more likely that the reduction in IFNγ is due to suppression of other immune cells in the pre-metastatic niche (not addressed in [26]).

T_{reg} cells are a specialized subset of $CD4^+$ T cells, capable of suppressing cells of both the innate and adaptive immune response. With the ability to limit the effectiveness of antitumor immune responses, this is another cell population potentially crucial but relatively understudied in the pre-metastatic niche. Through mechanisms not yet fully understood, MDSCs can promote the clonal expansion of T_{reg} cells and convert naïve $CD4^+$ T cells to T_{reg} cells [57–59], suggesting the accumulation of MDSCs in the pre-metastatic niche may indirectly suppress T and NK cell functions through the expansion of T_{reg} cells.

Regardless of the mechanism, there is strong evidence of reduced immune surveillance in pre-metastatic organs. Independent studies have shown treatment with conditioned media from both melanoma and hypoxic breast tumor cells increases metastatic burden in mice injected with LLC [8] and B16F10 melanoma [24] cells, respectively. As mentioned earlier, this

suggests firstly that the homing of tumor cells to specific organs is determined by the origin of the TDSFs, but also, that immune surveillance is reduced in these secondary organs to allow tumor cells of a different origin to the TDSFs in the conditioned media to colonize and grow. Hence, while very little is known regarding immune surveillance in pre-metastatic organs, its importance as a potential mechanism of pre-metastatic niche formation should not be underestimated.

7 Does the pre-metastatic niche control tumor cell dormancy?

Exactly when pre-metastatic niche formation is initiated during tumor progression has not yet been clearly defined. Multiple clusters of BMDCs have been shown to accumulate in the lungs between 7 and 14 days after injection of tumor cell-conditioned media in mouse breast and melanoma tumor models [8, 24], suggesting it can occur early during primary tumor growth in animal models (Fig. 2). Hypoxia occurs early during tumor progression, and secretion of hypoxic TDSFs capable of priming a pre-metastatic niche can also be expected to occur at this time (Fig. 2). Pre-metastatic niche formation most likely occurs as a consequence of and in parallel to formation of the primary tumor microenvironment (Fig. 2). TDSFs that promote the mobilization and accumulation of bone marrow progenitor cells at the primary tumor site would also direct them to pre-metastatic sites (Fig. 2). The consequences of pre-metastatic niche formation early during tumorigenesis may be further reaching than we know at the moment. DTCs can have less genetic aberrations than tumor cells at the primary site, indicating dissemination can occur early during tumor progression [2, 60]. In this case, the microenvironment of the secondary organs then becomes extremely important in controlling the fate of these DTCs. Creation of pre-metastatic niches means DTCs may not need to acquire all of the mutations necessary to complete the metastatic cascade and can instead rely on the pre-metastatic niche environment to make up for anything the tumor cell alone may lack in order to successfully metastasize (Fig. 2). Supporting the hypothesis that pre-metastatic niches might aid early DTC survival is the large group of patients diagnosed with cancers of unknown primary origin [61], that present with metastatic lesions in the absence of a corresponding primary malignancy. The ECM, angiogenesis, immune suppression, and hypoxia are all associated with either the initiation of tumor cells into, or the escape from, dormancy [60, 62–64]. Pre-metastatic niches could control the initiation of dormancy by pushing DTCs into a quiescent state until local or systemic changes make the metastatic environment suitable for outgrowth (Fig. 2). Once a conducive microenvironment is created, factors including VEGF, fibronectin, and MMPs secreted from myeloid cells in the niche promote the angiogenic switch necessary to allow tumor cell escape from dormancy [60, 63]. If the factors driving pre-metastatic niche formation cease, for example by removal of the primary tumor through surgery and adjuvant chemotherapy, the quiescent state of these dormant cells might be maintained until such time as conditions are again suitable for metastatic growth. Depending on the nature of the primary cancer, this could occur months or decades after successful treatment.

8 Exosomes as emerging coordinators of the pre-metastatic niche

Interactions with and between cells in the pre-metastatic niche have generally been assumed to occur through cell–cell contact or the release of soluble tumor-derived factors. However, with recent advances in the understanding of how exosomes mediate cellular communication in the primary tumor microenvironment, their involvement in pre-metastatic niche formation is also beginning to be uncovered. Exosomes are small membrane-bound vesicles, 50 to 100 nm in size, capable of mediating communication with surrounding cells or ECM components through cell surface receptor interactions or the horizontal transfer of their contents into recipient cells. Exosomes serve as delivery vehicles for mRNA, small RNAs, micro RNAs, and proteins under normal and pathological conditions, and have been purified from *in vitro* cultures of multiple cell types including primary cells of the immune and nervous system, fibroblasts, keratinocytes, epithelial cells, endothelial cells, mesenchymal stem cells, and, importantly, numerous tumor cell lines [65]. Exosome release is increased from tumor cells, with the number of exosomes secreted correlating to the malignancy of the tumor [66]. Tumors release exosomes into the surrounding microenvironment as well as into the bloodstream and have thus been considered as potential mediators of pre-metastatic niche formation [67]. The exact role exosomes play in the modification of pre-metastatic organs remains largely unknown; however, exosomes from melanoma cells have recently been described to reprogram bone marrow progenitor cells through the Met tyrosine kinase receptor, inducing a pro-vasculogenic cellular phenotype promoting vascular leakiness at pre-metastatic sites [68]. Furthermore, exosomes from highly metastatic cell lines compared to non- or poorly metastatic cell lines expressed significantly higher amounts of protein, while exosome protein concentration was demonstrated to also increase with stage in a subset of melanoma patients [68]. Exosomes have also been described to contribute to pre-metastatic niches in a process dependent on CD44v6, a marker of cancer-initiating cells in rat pancreatic adenocarcinoma [69], and through the enhancement of VEGFR1 expression and angiogenesis in the pre-metastatic niche when shed from $CD105^{+}$ human renal cancer stem cells [70].

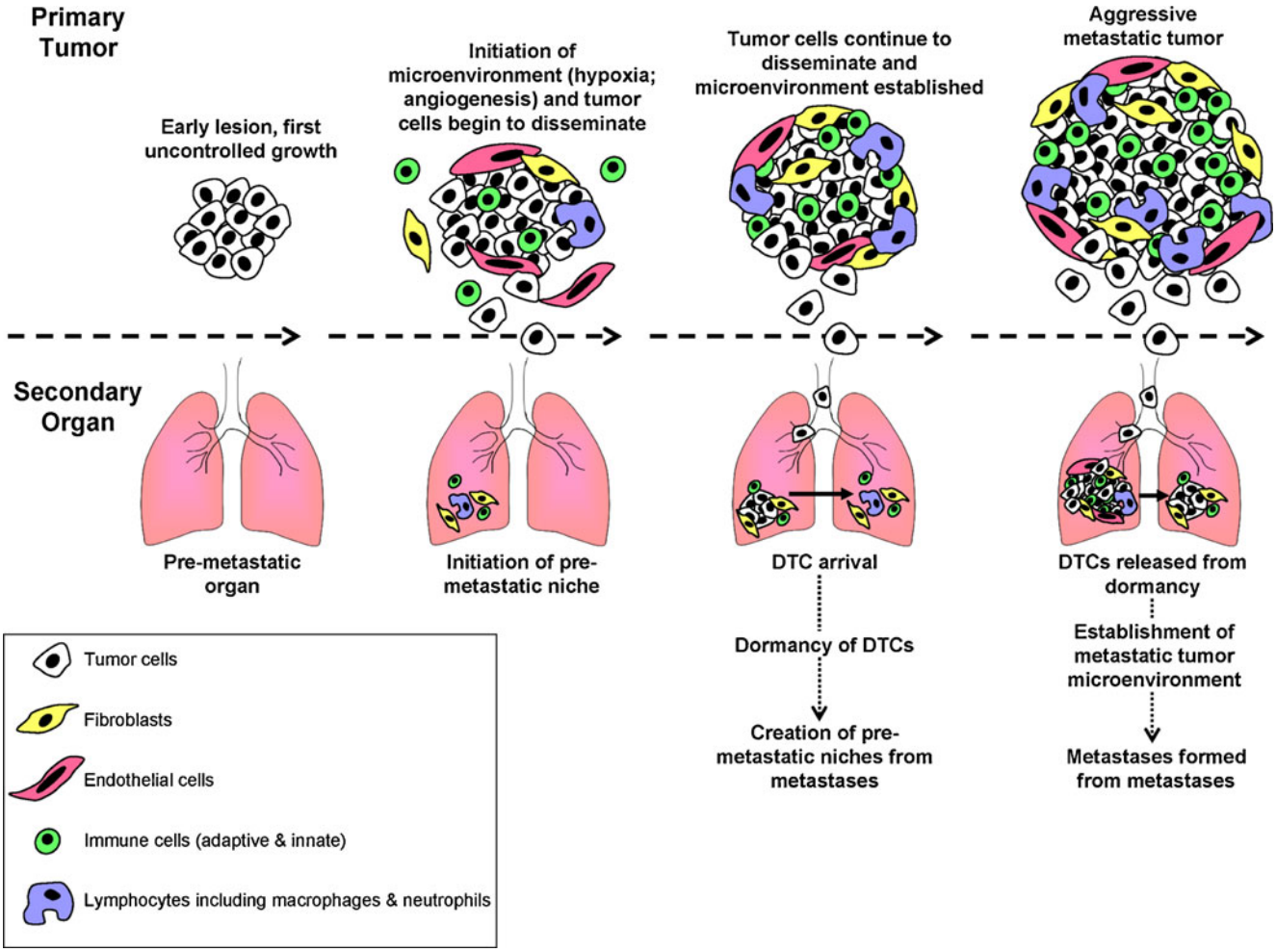

Fig. 2 Timeline of pre-metastatic niche development during primary tumor progression. Initial malignant lesions contain tumor cells undergoing uncontrolled proliferation. The primary tumor becomes hypoxic, which then promotes the mobilization and recruitment of local and bone marrow-derived cells including fibroblasts, endothelial cells, lymphocytes, and immune cells to create a supportive primary tumor microenvironment. In parallel, mobilized bone marrow progenitors also reach secondary organs, predisposing them as future sites of metastases (such as the lung in breast cancer). Early DTCs begin to arrive at these sites. Tumor cells continue to leave the primary site, and increasing numbers of DTCs reach pre-metastatic sites in secondary organs. Upon arrival, some DTCs survive and enter dormancy until a suitable microenvironment is established. The DTC-containing pre-metastatic niches can then promote further niche formation to allow tertiary tumor spread. The established primary tumor continues to promote pre-metastatic niche formation and release DTCs. Once the microenvironment at secondary sites is suitable, adept DTCs are released from dormancy and outgrow into metastatic lesions capable of promoting metastasis from metastases

Tumor-derived exosomes also have other functions that further implicate them as drivers of pre-metastatic niche formation. Firstly, tumor-derived exosomes suppress the function of DCs [71, 72], NK cells [73], and T lymphocytes [74, 75] through various mechanisms. Secondly, two recent studies have demonstrated a role for tumor-derived exosomes in the differentiation and mobilization of MDSCs. Xiang and colleagues reported that exosomes secreted by breast cancer cells could be taken up by bone marrow cells and promote their differentiation into $CD11b^{+}/Gr\text{-}1^{+}$ MDSCs, capable of modifying the tumor microenvironment and promoting tumor growth through the expression of COX2, IL-6, VEGF, and arginase1 [76]. Additionally, the MyD88-Toll-like receptor (TLR) signaling pathway has been demonstrated to be critical for tumor exosome-mediated expansion of these $CD11b^{+}/Gr\text{-}1^{+}$ MDSCs and induction of the proinflammatory cytokines that promote metastasis [77]. Significantly, several studies have also demonstrated the potent immunosuppressive functions of MDSCs expanded by tumor-derived exosomes, on both DCs [78] and T cells [75, 77].

Exosomes are emerging as important mediators of tumor progression, with more and more research focused on their roles in the primary tumor microenvironment. The ability of exosomes to promote tumor growth at both the primary site and secondary organs through immunosuppression of the adaptive immune system, either directly or through the

expansion of MDSC populations, is a critical function that needs to be investigated at the pre-metastatic niche as well. Inadvertently, exosome function in the pre-metastatic niche has most likely been observed already, because of the use of tumor cell conditioned media models to study its formation. The secretome of various tumor cell lines contains both soluble factors and exosome-associated proteins [79], suggesting that exosomes were most likely present in tumor cell conditioned media in previous models of the pre-metastatic niche. Therefore, we may, in the future, discover that some of the important processes in pre-metastatic niche formation, such as mobilization and differentiation of MDSCs, vascular remodeling, inflammation, immunosuppression, and even tumor cell dormancy, are consequences of tumor-derived exosomes and not soluble factors alone.

9 Is the pre-metastatic niche reversible?

Metastatic disease is the major cause of cancer-related morbidity and mortality. Once tumor cells have disseminated from the primary site, current treatments only help to prolong patient survival instead of completely curing patients of their disease. Formation of pre-metastatic niches in secondary organs before tumor cells have begun to metastasize may provide an explanation as to why metastatic disease has proved to be incurable so far. By preparing the microenvironment of secondary organs in advance, it is not necessary for primary tumor cells to acquire extra mutations to allow colonization and proliferation in secondary sites, as pre-metastatic niches provide the additional support. While understanding the processes that drive pre-metastatic niche formation furthers our understanding of metastatic progression, it also poses new challenges in the search to develop a cure for metastatic disease. Surgery to completely remove the primary tumor at an early stage still seems to be the most effective prevention of metastatic progression. Maintenance of the pre-metastatic niche appears to require ongoing production of factors by the primary tumor, with the number of $CD11b^+$ myeloid cells in pre-metastatic lungs shown to decrease daily after the cessation of treatment with tumor conditioned media [43]. However, in those patients whose tumors could not be removed completely or in a timely manner, pre-metastatic niches would be maintained by the remaining primary tumor or may already have done their job in supporting tumor cell colonization. Once a tumor cell has established itself in a secondary organ, continued production of pre-metastatic niche-promoting factors by the primary tumor may not be required, as established metastatic tumors could then support formation of further metastases. Therefore, targeting pre-metastatic niches to reduce or prevent metastatic disease in these patients, and not relying on complete removal of the primary tumor alone, would be highly desirable.

Biomarkers of the pre-metastatic niche. TDSFs identified in pre-metastatic niche formation vary depending on the tumor model in question, the metastatic characteristics of the tumor cell lines, and conditions such as hypoxia within the primary tumor itself. Targeting specific TDSFs such as LOX and LOXL proteins [21, 80], MCP-1 [24], G-CSF [16], and VEGF [8] has been shown to decrease metastasis by reducing pre-metastatic niche formation. Yet, because different TDSFs drive different processes in the pre-metastatic niche even within the one model, treatment targeting just one TDSF may only be partially effective. Therefore, treatment would most likely require drugs against multiple TDSFs per patient for different cancers, and rely on a clear distinction between those TDSFs which are functionally critical *versus* functionally redundant to pre-metastatic niche formation in each cancer type. It may be more effective to target pathways whose activation results in production of multiple pre-metastatic niche-promoting factors from either the tumor itself such as the hypoxic response pathway and Hif-1α [80], or the S1PR1-STAT3 [43] and TLR [27, 81] signaling pathways in myeloid cells in pre-metastatic organs.

Additionally, TDSFs could be used as biomarkers of pre-metastatic niche formation. Pre-metastatic niche "signatures" using TDSFs as biomarkers could allow patients to be stratified depending on their individual risk of developing metastatic disease. This would allow treatment and monitoring of each patient to be altered according to their likelihood of metastatic relapse. Therefore, patients with an increased risk could be treated more aggressively earlier in their disease course, while those patients with a lower risk could avoid unnecessary treatment.

Exosomes also show potentially more promise as pre-metastatic niche biomarkers. The qualitative differences in the exosomal content of proteins, mRNA, and miRNA correlate with metastatic potential and pre-metastatic niche formation *in vivo* [68]. In addition, the amounts of tyrosinase-related protein-2, very late antigen-4 (VLA-4), heat shock protein 70, and the MET oncoprotein found in circulating exosomes from melanoma patients comprise an exosomal signature to predict stage, prognosis, and survival for patients with metastatic melanoma [68]. RNA isolated from microvesicles secreted by human glioblastoma (GBM) cells [82] and exosomes purified from the serum of GBM patients [83] can distinguish and provide information for the diagnosis and treatment of GBM. While the differential expression of exosome-derived Survivin, an inhibitor-of-apoptosis family member, also shows potential as an early detection biomarker in prostate cancer [84]. Moreover, exosomes show great potential for treatment as well. Antigens carried by exosomes have increased immunostimulatory capacity, leading to investigations into their potential as "vaccines" in cancer [65]. Exosomes derived from heat-stressed tumor cells, for example, induce a more potent antitumor

immune response through the chemoattraction and stimulation of DCs and T cells [85].

Targeting MDSCs and immunosuppression in the pre-metastatic niche. Targeted therapeutics with proven efficacy in mouse models, and more importantly in the clinic, have been developed for specific subsets of MDSCs. These are well described in [36] and will not be addressed in detail here. MDSCs have been therapeutically targeted with drugs that prevent subset expansion and proliferation in the bone marrow as well as their mobilization and recruitment to other organs, induce apoptosis, overcome the block maintaining their immature state, and inhibit the mechanisms of immunosuppression [36]. As discussed earlier, the immunosuppressive function of MDSCs in the context of the pre-metastatic niche has not been explored in great detail, but could be a key factor limiting the overall success of cancer immunotherapy in the treatment of metastatic disease. While the different subsets of MDSCs do not share many overlapping mechanisms of immunosuppression, the functional changes in the MDSC subpopulations can be mediated by common TDSFs, and potentially exosomes. Therefore, targeting common effector molecules may be more successful than targeting suppressive pathways unique to specific subpopulations of MDSCs.

Cancer immunotherapy has not yet been utilized as a potential treatment to prevent pre-metastatic niche formation, but is worth investigating. Reduced NK cell cytotoxicity, for example, has been reported in tumor-bearing mice and human cancer patients [86], and is associated with late-stage disease in myeloma [87], melanoma [88–90], and lung cancer [88]. While human studies are limited by the availability of samples from patients with early-stage cancer, reduced NK cell cytotoxicity has also been reported in the pre-metastatic phase in murine breast cancer models [24], suggesting suppression could occur early in tumorigenesis. Treatment regimes, such as adoptive transfer of NK cells early during disease, may prove beneficial in preventing metastatic relapse through recovery of NK cell activity in distant organs. Adoptive transfer of human NK cells cultured and activated *in vitro* has been used, with varying degrees of success, in the treatment of different forms of leukemia (reviewed in [91]). Other potential therapeutic approaches include redistributing cytotoxic NK cells [92], boosting NK cell activity by upregulating activating ligands (such as NKG2D) on tumor cells using chemotherapeutics as demonstrated in multiple myeloma [92], or through the use of monoclonal antibodies targeting tumor cells to enhance antibody-dependent cell-mediated cytotoxicity [86, 93]. Enhanced NK cell function may prove beneficial not only at pre-metastatic organs, but also systemically by eliminating metastasizing tumor cells in circulation, prior to their arrival at pre-metastatic sites.

If MDSCs are indeed responsible for the reported reduced cytotoxicity of NK cells in the pre-metastatic niche [24, 46], treatment aimed at boosting NK cell activity alone will not be as effective while suppressive MDSCs still populate pre-metastatic sites. Therefore, combination therapy to target the suppressive function of MDSCs and boost NK cell function may be more efficacious in restoring normal immune surveillance to the pre-metastatic niche. This idea could also be applied to $CD8^+$ T cells, if proven to play a role in pre-metastatic niche formation.

10 Conclusions and perspective

Formation of pre-metastatic niches in ectopic organs, driven by the primary tumor, is now a well-established process promoting metastatic progression (Fig. 1), yet many unanswered questions still remain regarding the exact mechanisms of their formation. Do all tumors set up pre-metastatic niches in specific organs, or are they only formed in the case of highly malignant or hypoxic primary tumors? Do pre-metastatic niches persist after their source of TDSFs is removed, and if so, for how long? To what degree does immunosuppression of the innate and adaptive immune responses play a role in pre-metastatic niche formation? Does pre-metastatic niche formation regulate tumor cell dormancy and is this changed by removal of the primary tumor? Are metastatic lesions capable of creating pre-metastatic niches and are the mechanisms similar to or different from those utilized by the primary cancer? Can TDSFs or exosomes be utilized as biomarkers of pre-metastatic niche formation and therefore predict metastatic progression? What is the therapeutic window in which the pre-metastatic niche can be targeted, if at all? Are MDSCs viable targets for treatment or prevention of pre-metastatic niche formation? More sophisticated models and techniques will need to be developed to answer these questions regarding pre-metastatic niche development, maintenance, and treatment.

Exactly how the primary tumor promotes formation of these pro-metastatic environments at distant sites is still under scrutiny, but is crucial to understanding metastatic progression. Treatment of metastasis in the clinic will remain unsuccessful, and secondary disease will remain the cause of most cancer-related fatalities while pre-metastatic niches persist. Better targets for treatment of metastatic disease need to be found. Increased understanding of the mechanisms driving pre-metastatic niche formation will aid in the discovery of novel targets and may finally provide the elusive cure for metastatic disease.

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