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Organotropism in Breast Cancer Metastasis: How the Pre-Metastatic Niche Shapes Organ-Specific Spread

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ABSTRACT

Metastasis is a major cause of mortality in patients with advanced breast cancer and is influenced by the ability of tumor cells to establish supportive microenvironments in distant organs. A major contributor to metastatic progression is the formation of the pre-metastatic niche (PMN), in which distant organs are remodeled before the arrival of metastatic cells. This process represents a potential window for therapeutic intervention before the establishment of metastatic disease. This review synthesizes information from primary studies and literature reviews to analyze how the primary tumor uses exosomes and other secreted factors to develop organ-specific PMNs by remodeling their microenvironments. We highlight evidence that organotropism is influenced by the molecular composition of tumor-derived exosomes, including their integrin profiles, which can promote interactions with specific target organs. Exosomal contents such as lncRNAs and secreted growth factors can also activate resident fibroblasts and promote extracellular matrix remodeling, including changes in collagen deposition and cross-linking. In parallel, tumor-derived factors can reprogram myeloid-derived suppressor cells, neutrophils, and T-cells to create an immunosuppressive environment, while distinct exosomal miRNAs, lncRNAs, and proteins can promote angiogenesis and alter vascular permeability. Together, these mechanisms demonstrate that PMN formation is not a single pathway but involves coordinated changes across multiple cellular and molecular components in distant organs. Where mechanisms discussed occur after tumor-cell arrival rather than before, this distinction is marked explicitly. These findings also highlight the potential of circulating exosomal cargo as a source of biomarkers for early detection of metastatic risk and identification of organ-specific molecular signatures, although most mechanisms reviewed derive from preclinical models, and substantial clinical validation is required before such approaches can be applied in practice.

INTRODUCTION

Breast cancer is currently the most commonly diagnosed cancer globally, after surpassing lung cancer in 2020. In the same year, it accounted for 11.7% of all cancer cases, with an estimated 2,261,419 new cases and 684,996 deaths, which is the fifth-highest of any cancer (Sung et al., 2021). Metastasis, the spread of cancer cells beyond the primary site to distant organs, converts a treatable local disease into a systemic one, and over 90% of breast cancer deaths result from metastatic complications (Jin & Mu, 2015). Some

studies estimate that 6–10% of patients present metastatic disease at diagnosis and that around 20–30% of patients initially diagnosed with localized disease eventually progress to stage IV (R. Wang et al., 2019). Treatment strategies for breast cancer vary widely and are selected based on tumor subtype, stage, and metastatic status (Jin & Mu, 2015). Common treatment approaches include surgery, chemotherapy, radiotherapy, endocrine therapy, and immunotherapy, with emerging forms of treatment, including gene therapy, currently under investigation. For localized, non-metastatic disease, surgery remains the most common form of treatment (J. Wang & Wu, 2023), while metastatic disease is managed with systemic therapies that aim to control rather than cure the cancer. The poor prognosis of metastatic breast cancer patients highlights the limitations of current treatment strategies, which are mostly reactive, targeting metastasis only after it has already occurred. This underscores the need for a deeper understanding of the molecular mechanisms of metastasis, with a focus on the early stages that enable the migration of tumor cells to distant organs.

One of the earliest steps in the metastatic cascade is the formation of a pre-metastatic niche (PMN), a hospitable environment created in distant organs that provides favorable conditions for tumor cell colonization and survival (Doglioni et al., 2019). For the sake of this review, the PMN is defined as a set of changes made to a distant organ before the arrival of any disseminated tumor cells. This temporal criterion is applied throughout the study: a mechanism is qualified as forming a PMN only if it is capable of occurring independently of, and prior to, tumor-cell arrival. Research has demonstrated that the formation of a PMN follows Paget's 'Seed and Soil Theory', where the primary tumor creates a 'soil' (the pre-metastatic niche) in which the 'seed' (disseminating tumor cells) can grow (Kaplan et al., 2005). Tumors create PMNs by secreting a variety of factors that alter the microenvironment of the target site, priming it for colonization. These factors include angiogenic factors and pro-inflammatory cytokines, as well as tumor-derived extracellular vesicles called exosomes, which are the key mediators of PMN establishment (Y. Liu & Cao, 2016). Exosomes are small, membrane-bound extracellular vesicles that carry soluble proteins, lipids, or nucleic acids as chemical cargoes, communicating with adjacent cells or distant organs (S. Huang et al., 2022). Studies have shown that eliminating and inhibiting molecules and cells aimed at forming a PMN, such as VEGFR1+ pre-metastatic cell clusters in Lewis Lung Carcinoma, prevented PMN formation and markedly reduced metastasis (Kaplan et al., 2005). This suggests that inhibiting the PMN may represent a viable strategy to control metastasis and also be a good preventive strategy to halt further progression of the cancer.

Tumor-derived exosomes are therefore attractive both as biomarkers that could predict metastasis and as targets whose inhibition might prevent PMN formation before metastases arise (Doglioni et al., 2019; S. Huang et al., 2022). This review aims to compare and analyze the mechanisms through which breast cancer tumors create pre-metastatic niches across certain organs through exosomes, influence the structural integrity and permeability of the stroma, suppress the immune microenvironment of the target organ, and induce permeability and angiogenesis within resident vasculature. This review will help provide a better understanding of how tumor-derived factors, particularly exosomes, coordinate changes in the stromal, immune, and vascular components of distant organs to establish organ-specific PMNs.

Because several mechanisms in the literature span both phases of metastasis, this review distinguishes throughout between pre-metastatic niche formation, as defined above, and metastatic colonization, which will refer to the changes made to enhance extravasation, survival, and proliferation occurring after tumor cells have already reached the target organ. Such mechanisms are retained in the review as both pre-arrival and post-arrival changes are often mediated by the same signaling molecules and cell populations. However, the distinction is maintained throughout so that the evidence for each phase is clear. Whenever a mechanism involves both phases, or when the evidence primarily focuses on metastatic colonization, it is explicitly stated.

METHODOLOGY

The literature for this review was selected through searches of databases such as PubMed, Google Scholar, and ScienceDirect, between June 2026 and September 2026. Multiple search terms were used in combination, including "breast cancer metastasis", "pre-metastatic niche", "organotropism", "tumor-derived exosomes", "extracellular vesicles", "extracellular matrix", "myeloid-derived suppressor cells", "regulatory T-cells", "tumor-associated neutrophils", "blood-brain barrier", and "angiogenesis". Studies were only considered if they met the following criteria: (i) they investigated the mechanisms of pre-metastatic niche formation or organ-specific metastasis; (ii) they directly examined breast cancer metastasis, or demonstrated mechanisms that were tested on breast cancer models; (iii) they were published in reputable, peer-reviewed journals that were in English; (iv) the full text of the paper was accessible. While no lower data limit was applied, due to many of the foundational papers of the field being several decades old, studies published after 2010 were strongly preferred.

Papers were excluded if they examined only post-arrival tumor growth without any reference to pre-metastatic niche formation, demonstrated mechanisms without any relevance shown to breast cancer, or displayed mechanisms only at the surface level without sufficient explanation of the molecular and cellular interactions causing them. Additionally, priority was given to studies which provided causal evidence, such as through genetic knockout or pharmacological inhibition, over studies which only provided correlative evidence.

ORGANOTROPISM AND ORGAN-SELECTIVE PRE-METASTATIC NICHE FORMATION

What Is Organotropism?

When cancer metastasizes, tumor cells spread to distant organs in a non-random, organ-selective manner, a phenomenon known as organotropism. This is readily apparent in breast cancer, where Wu et al. (2017) studied 17,445 patients with distant metastatic breast cancer and revealed that the most common sites of metastasis were the bones, lungs, liver, and brain (50.7%, 24%, 19.7%, and 5.7%, respectively). In addition, an autopsy-based study by Budczies et al. (2015) analyzed 1,016 autopsied bodies with

metastatic cancers and found that the patients with breast cancers tended to have metastases in the liver, bones, non-regional lymph nodes, and lungs (80%, 79%, 60%, and 54%, respectively). The use of an autopsy-based dataset helps uncover data about clinically silent metastases, enabling more accurate metastatic rate estimations. Organotropism can be determined by intrinsic tumor-cell properties or by host and microenvironmental factors. The majority of invasive breast cancers are classified as either invasive ductal carcinomas (IDCs) (80-90%) or invasive lobular carcinomas (ILCs) (~10%), and both subtypes have been shown to display different organotropisms. Arpino et al. (2004) evaluated clinical and biological features of 4,140 patients with ILC and 45,169 patients with IDC, including the first metastatic site. They revealed that both subtypes have vastly different tropisms, with IDCs tending to metastasize to the lungs (17.6%), bones (35.5%), and distant lymph nodes (22.0%), while ILCs had a much higher relative tendency to metastasize to the peritoneum, gastrointestinal (GI) tract, and ovaries (6.7% vs. 1.8%).

A common mechanism by which cancer cells communicate with target organs to form the PMN is through exosomes. While exosomes contain a variety of proteins, lipids, and genetic material, integrins have been identified as key determinants of metastatic organotropism. Hoshino et al. (2015) showed in mouse models that inhibition of integrin β4 and β5 expression via short hairpin RNAs or blocking their binding by HYD-1/RGD peptides reduced uptake of exosomes by organ-resident cells and also decreased the rate of lung and liver metastasis. Tumor-derived exosomes also vary in composition and in the cell types they target according to the destination organ. Breast cancer patients with lung metastases had higher exosomal integrin β4 levels, while pancreatic patients with liver metastases had higher levels of exosomal integrin αv, compared to patients without those metastases (Hoshino et al., 2015). Additionally, Hoshino et al. (2015) showed that lung-tropic (LuT) breast cancer exosomes mainly targeted fibroblasts and surfactant protein C (SPC)-positive epithelial cells, while liver-tropic pancreatic cancer exosomes mostly fused with Kupffer cells. Chen et al. (2018) reported that breast cancer liver-tropic exosomes carry integrin αvβ5, while LuT exosomes carry integrins α6β4 and α6β1 and bind lung-resident fibroblasts and epithelial cells to prime the PMN. They also showed that inhibiting these integrins decreases exosomal uptake and reduces metastasis.

Once the exosome interacts with the target organ, it triggers the formation of the PMN. Integrins interact with the extracellular matrix (ECM) of the target organ, leading to vascular leakiness and promoting the uptake of exosomes by parenchymal cells. Hoshino et al. (2015) discovered that 4175-LuT exosomes, expressing integrins α6β4 and α6β1, co-localized with S100A4+ lung fibroblasts in laminin-rich lung microenvironments. Additionally, they revealed that 4175-LuT exosomes promoted vascular leakiness within 24 hours of injection, before exosomal uptake by parenchymal lung cells. Their study demonstrated that 24 hours after priming mice with 4175-LuT-derived exosomes, the lungs exhibited increased metastatic capacity and even redirected bone-tropic 1833-BoT cells to the lungs. This demonstrates how certain exosomes can facilitate and increase organ-specific metastatic capability, and can even redirect cancer cells with other tropisms to the target organ.

Organ-Specific Differences in PMN

Tumor-derived exosomes employ different mechanisms to establish a PMN based on the target organ (Table 1). These organ-specific pathways vary depending on whether the local tissue is more or less permissive to colonization. In bone, tumor-secreted factors mainly focus on remodeling the microenvironment rather than overcoming inhibitory signals. The primary tumor initiates a 'vicious cycle' in bone metastasis, in which it releases tumor-derived factors such as osteopontin (OPN), parathyroid hormone-related peptide (PTHrP), heparanase, IL-1, IL-6, and prostaglandin E2 (PGE2), which enhance osteoclast formation, leading to enhanced bone resorption. Resorption in turn releases matrix-bound growth factors (IGF1, PDGF, TGFβ) and calcium that drive tumor proliferation (Weilbaecher et al., 2011). This creates a self-reinforcing cycle, where secreted factors from tumors cause bone resorption, which causes the release of growth factors. These growth factors then allow the cancer cells to proliferate, which further causes a greater amount of bone-degrading secreted factors to be released. It is important to note that this mechanism is not strictly pre-metastatic, as the self-reinforcing component requires tumor cells to be present in bone. The pre-metastatic elements of the cycle (E-selectin and SDF-1 priming) are described in Section 3.3.

In contrast, the brain is comparatively less permissive to metastasis, so colonization depends on mechanisms that circumvent this hostility. Valiente et al. (2014) identified that the brain contains plasmin, an inhibitor of metastatic invasion. Plasmin reduces the adherence of circulating tumor cells (CTCs) to resident cells by converting membrane-bound astrocytic Fas ligands into a paracrine death signal for cancer cells. It also inactivates the neuronal cell adhesion molecule L1, which would otherwise promote the spread and formation of large metastases. To overcome plasmin, brain-tropic cells release serpins (neuroserpin, serpin B2) that promote cancer cell survival and vascular co-option (utilization of pre-existing vasculature rather than inducing new vessels) in the brain.

Exosomes also vary their PMN-forming mechanisms based on the level of barrier protection within the target organ. In bone, tumor cells extravasate readily because much of the marrow vasculature is fenestrated and sinusoidal, thereby making it more permeable (Ramasamy, 2017). This allows them to prioritize rebuilding the bone microenvironment to make it habitable for the secondary tumor through processes such as osteomimicry, which improves homing, adhesion, proliferation, and survival in the bone microenvironment, rather than requiring specific mechanisms to bypass barriers (Chen et al., 2018). In contrast, the blood-brain barrier (BBB) is highly regulated and requires specific exosomal mechanisms to induce extravasation across the non-fenestrated capillaries within the brain, increasing BBB permeability and allowing trans-endothelial migration of CTCs. As reviewed by Chen et al. (2018), exosomes containing CD44, VEGF, and CXCR4 can enhance this transendothelial migration process by disrupting endothelial integrity. Additionally, Angiopoietin-2 (Ang-2), secreted by the primary tumor, increases BBB permeability by impairing ZO-1 and claudin-5 tight junction proteins and can cause the subsequent colonization of triple-negative breast cancer (TNBC) cells in the brain (Chen et al., 2018).

Table 1. Organ-specific barrier characteristics and central pre-metastatic niche mechanisms in breast cancer metastasis.

Organ	Frequency (Wu et al., 2017)	Barrier Type	Central Mechanism	Evidence Type	PMN or Colonization	Interpretive Permissiveness	Citation
Bone	50.7%	Fenestrated, sinusoidal endothelium	Tumor-secreted PTHrP drives osteoclastogenesis and bone resorption, releasing matrix-bound growth factors.	Review of mouse and human studies	Primarily colonization; pre-metastatic component limited to systemic priming	Broadly permissive	(Weilbacher et al., 2011)
Liver	19.7%	Fenestrated vasculature, no basement membrane	Exosomal integrin αvβ5 taken up by Kupffer cells, generating a fibronectin-rich microenvironment	Mouse models with patient exosome data	Pre-metastatic	Permissive	(Hoshino et al., 2015; Martin et al., 2010)
Brain	5.7%	Blood-brain barrier; non-fenestrated capillaries	Ang-2, CD44, VEGF and	Review of preclinical studies	Mixed, as barrier conditioning is	Restrictive	(Chen et al., 2018)

			CXCR4 increase permeability and transendothelial migration; serpins counteract plasmin		pre-metastatic, while serpin-mediated survival is post-arrival		
Lung	24%	Alveolar-capillary basement membrane	Hypoxia-induced LOX cross-links collagen IV and recruits CD11b+ myeloid cells	Mouse xenografts with human tissue correlation	Pre-metastatic	Permissive	(Erler et al., 2009)
Regional Lymph Nodes	Not classified as distant metastasis	Lymphatic sinuses, low resistance	VEGF-C induces lymphangiogenesis, expanding routes for continued dissemination	Mouse models	Pre-metastatic	Permissive	(Hirakawa et al., 2007)

Note: Permissiveness designations are interpretive summaries, intended to highlight broad differences in receptivity. Metastatic permissiveness is not an established categorical classification and is context-dependent, with many organs showing both permissive and restrictive features.

Why Are Specific Organs Targeted First?

Although breast cancer can spread to almost any organ, it characteristically seeds certain organs first. The mechanisms discussed in this section concern where tumor cells first arrive and arrest, rather than how the receiving tissue forms a PMN, with the exception of bone. However, it is included because the organ sequence of metastasis nevertheless constrains where a PMN can form. Regional lymph nodes (RLN) are the most common first site of metastasis. For example, Luo et al. (2023) studied 256,504 breast cancer patients and noted a 25.57% incidence rate of RLN metastasis across all patients, displaying the high prevalence of lymphovascular invasion (LVI) in patients both with and without distant metastases.

RLNs are commonly the first site involved because of their proximity to the primary tumor and position along the natural route of systemic spread. Nathanson et al. (2018) analyzed data from breast cancer patients over 24 years, investigating whether LVI favors lymphatic or blood vessels. They found that tumor cells are more likely to invade peritumoral lymphatics because blood capillaries have interendothelial tight junctions, pericytes, and basement membranes, which act as mechanical barriers to cancer cell invasion, features that are not seen in lymphatic vessels. This suggests that CTCs are more likely to disseminate via the lymphatic than the vascular route. Apart from their close proximity to the primary tumor, RLNs also tend to be the first checkpoint within the metastatic cascade where cells access the systemic circulation. Metastasis to regional lymph nodes effectively tripled the incidence of subsequent distant metastasis in patients with invasive breast cancer containing LVI (Nathanson et al., 2018). This indicates lymph-node involvement in breast cancer metastasis is a significant marker of dissemination risk.

Bone is the most common first distant site for all breast cancer subtypes except the basal-like subtype, with Kennecke et al. (2010) reporting that in the luminal B, luminal/HER2, and HER2 breast cancer subtypes, cumulative bone metastasis exceeded 30%. The perisinusoidal marrow, which expresses high levels of E-selectin and SDF-1, is a favorable homing site for CTC adhesion. Using high-resolution imaging of calvarial breast cancer xenografts, Price et al. (2016) found that CTCs engaged the endothelium only in sinusoidal and perisinusoidal regions, where E-selectin and SDF-1 are high, with E-selectin mediating homing and SDF-1 anchoring the cells. CTCs expressed multiple E-selectin ligands, and the E-selectin inhibitor GMI-1271 blocked their entry into bone, highlighting E-selectin's essential role in homing.

The lung is the other major first site and the most frequent site for the basal-like/triple-negative subtype. Unlike bone, which is favored for its biochemical microenvironment, the lung is favored for anatomical and mechanical reasons. While a site's compatibility depends on molecular interactions, the delivery and arrest of CTCs is governed largely by an organ's mechanical properties (Azevedo et al., 2015). While in lymphatic invasion, RLNs are the first inhabitable organ in the pathway, the lungs are the first inhabitable organ for CTCs from breast cancer in vascular circulation and act as the first major bottleneck in their pathway (Medeiros & Allan, 2019). Because of the lungs' central role in the vascular system, all CTCs within any form of vasculature will pass through the lungs, which does not occur in other distant organs.

Medeiros & Allan (2019) observed that the average size of CTCs is approximately 5 times the size of pulmonary capillaries, which substantially increases the likelihood of arrest within the capillary beds and subsequent extravasation. Together, these factors make the lung one of the most commonly seeded organs in breast cancer.

STROMAL AND EXTRACELLULAR MATRIX REMODELING IN THE PRE-METASTATIC NICHE

Remodeling of Resident Fibroblasts

Fibroblasts maintain the ECM but are also central to PMN formation, secreting factors that remodel the microenvironment to make it receptive to metastatic cells. Studies appear to disagree, however, on the baseline state of fibroblasts before PMN formation and the mechanisms driving their conditioning. Tang et al. (2025) demonstrated in mouse models that lung fibroblasts are normally quiescent and require activation through exosomal cargo: resident lung fibroblasts take up the long non-coding RNA lnc-PDGFD and differentiate into cancer-associated fibroblasts (CAFs) via Y-box binding protein 1 (YBX1) signaling, with activation defined by the acquisition of CAF-associated markers. In contrast, Gong et al. (2022) used single-cell RNA sequencing in mouse models to identify a subpopulation of lung fibroblasts that are intrinsically immunosuppressive, enabling them to produce prostaglandin E2 (PGE2), which inhibits dendritic cells and suppresses monocytes. Here, tumor-derived factors do not initiate PGE2 expression but rather amplify it within pre-existing pathways. Tang et al. (2025) define fibroblast activation structurally, through differentiation into CAFs, while Gong et al. (2022) define it functionally, through their capacity to suppress immune cells by PGE2 production. In theory, a fibroblast can remain quiescent while still being immunosuppressive, showing that the two conclusions by the studies display different properties of the same cells, rather than opposing baseline states. Consistent with this interpretation, both studies agree that fibroblasts upregulate inflammatory cytokines essential for PMN establishment, including IL-11, CXCL12, CXCL8, and IL-1β. Tang et al. (2025) identified IL-11 as the predominant secreted cytokine, emphasizing its pro-fibrotic role in establishing a microenvironment suitable for metastasis. Gong et al. (2022) highlighted IL-1β as a critical pro-inflammatory cytokine: IL-1β stimulation triggers resident lung fibroblasts to express prostaglandin-endoperoxide synthase 2 (PTGS2), which encodes cyclooxygenase-2 (COX-2). COX-2 drives fibroblasts to reprogram myeloid cells into a dysfunctional, immunosuppressive state via PGE2 production. PGE2 in turn suppresses anti-tumor immunity by inhibiting MHC-I and MHC-II expression, decreasing the likelihood of a T cell response against disseminating tumor cells. Consistent with this, genetic ablation of PTGS2 in fibroblasts increased MHC-I and MHC-II on lung-resident dendritic cells and enhanced antigen uptake. Additionally, pharmacological inhibition of PGE2 largely restored anti-tumor immunity in the lungs and increased immunotherapeutic efficacy against lung metastases.

Beyond cytokine-mediated effects, tumor-derived extracellular vesicles can also alter fibroblast activity and ECM composition. Breast cancer-derived exosomes have been shown to contain high levels of caveolin-1 (Cav-1), a membrane transport protein that regulates vesicle transport and cell signaling

pathways. Y. Wang et al. (2023) tested this using breast cancer mouse models. In the experiment, orthotopic breast cancer mouse models with elevated levels of Cav-1 exhibited significantly greater lung metastasis, while Cav-1 inhibition reduced metastatic burden, confirming its role as a facilitator of metastasis. The study also demonstrated that Cav-1 promotes metastasis by upregulating the expression and deposition of key ECM proteins, including fibronectin (FN), nidogen, emilin1, and tenascin-C (TnC). Among these proteins, TnC, in particular, plays an important role in metastatic colonization (Lowy & Oskarsson, 2015). TnC directly activates Notch and Wnt signaling pathways within disseminated tumor cells. This maintains their pro-survival and stem-like properties and enables proliferation and cellular repair.

As with POSTN, these effects act on cells that are already within the tissue, with the pre-metastatic component, the Cav-1-driven ECM deposition, being described above. Interestingly, TnC also exhibits anti-adhesive properties that may promote tumor-cell motility rather than directly preventing CTCs from embedding themselves within the ECM. TnC achieves this by modulating the function of integrins and preventing their interaction with adhesive ECM components such as fibronectin. This reduced adhesion stimulates CTC motility and promotes deeper invasion into the tissue.

Apart from exosomes, cancer stem cell (CSC)-derived secreted factors reprogram resident fibroblasts. CSCs secrete growth factors such as TGF-β3 and TGF-β2 that can restore the expression of certain stromal components that were previously downregulated. CSCs also rely on various localized growth factors to proliferate and maintain their stem-like properties. A key example is periostin (POSTN), an extracellular matrix protein normally restricted to stem cell niches such as the mammary gland but largely downregulated in most tissues after adulthood. However, Malanchi et al. (2012) identified that POSTN was upregulated in primary lung fibroblasts following exposure to tumor-derived TGF-β3 and TGF-β2. Notably, this mechanism operates mainly after tumor cells arrive, as POSTN upregulation is induced by the infiltrating CSCs themselves. Therefore, it's a niche-maintaining mechanism rather than pre-metastatic conditioning, but is included here as the same fibroblast population is recruited at both stages.

Similar to TnC, POSTN promotes Wnt signaling, which is an essential pathway for stem cell maintenance in both mammary glands and tumors. POSTN binds Wnt1 and Wnt3 ligands, inducing Wnt pathway activation. Notably, POSTN was found to be indispensable for this process, as Wnt signaling activity was abrogated in its absence. Further, the establishment of CSC cultures was not possible in POSTN-deficient tumor models, and in vivo studies showed that CSCs failed to proliferate in POSTN-deficient pulmonary fibroblasts. Moreover, POSTN-deficient animals displayed reduced CSC populations. These findings establish POSTN as an essential niche factor that supports CSC growth and maintenance.
Collectively, resident fibroblasts undergo multiple layers of conditioning through exosomal uptake, cytokine signaling, and growth-factor stimulation that upregulate immunosuppressive pathways, remodel ECM composition, and support CSC maintenance to promote metastatic colonization.

Role of ECM in PMN Formation

As discussed, fibroblasts condition the ECM, but tumor-secreted enzymes and growth factors also remodel it directly to promote metastatic success. A key tumor-derived mediator of ECM remodeling is lysyl oxidase (LOX), an enzyme secreted by hypoxic breast cancer cells. LOX is transported to pre-metastatic sites, where it catalyzes cross-linking between collagen-IV and elastin within the basement membrane in mouse xenografts (Erler et al., 2009). This cross-linking increases ECM rigidity, enhancing the recruitment and adhesion of bone marrow-derived cells. This process recruits CD11b+ monocyte-derived cells, which in turn recruit CD11b+Gr-1+ myeloid cells. In human breast cancer cell-implanted mice, CD11b+Gr-1+ myeloid cells suppress anti-tumor immunity by inhibiting certain T cells, B cells, and natural killer (NK) cell-mediated immune responses (L. Yang et al., 2010). This immunosuppression substantially increases the metastatic and proliferative capacity of CTCs. Additionally, CD11b+Gr-1+ myeloid cells promote angiogenesis by incorporating into the endothelium, further supporting vascular development within the emerging niche (Erler et al., 2009).

While LOX cross-links and rigidifies the ECM, CD11b+ cells simultaneously express matrix metalloproteinases (MMPs) that cleave collagen into collagen peptide fragments. These collagen peptides possess chemoattractive properties and can contribute to the recruitment of bone marrow-derived cells (BMDCs), including populations expressing growth factor receptors such as VEGFR-1. These receptors can promote local inflammation and enhance CTC adherence and invasive capacity (Kaplan et al., 2005). Further MMP-mediated collagen degradation, along with LOX-mediated cross-linking, creates an ECM with both increased rigidity and enhanced permeability. Together, these findings show how CD11b+ cell-driven mechanisms, ECM cross-linking via LOX, and ECM degradation via MMPs influence the adhesive and invasive properties of the lung microenvironment.

In addition to ECM remodeling, tumor-derived factors also condition the vascular ECM to facilitate CTC extravasation into the lung tissue. Y. Huang et al. (2009) studied the effects of B16/F10 melanoma cell implantation on pulmonary vascular ECM integrity in nude mice. They found that after implantation, the integrity of the tunica intima, the innermost layer of blood vessel walls, was significantly disrupted. This disruption led to fibrinogen deposition and fibrin formation in the lungs, thereby increasing tumor cell adhesion. The study also showed that certain growth factors and enzymes, including angiopoietin-2 (Ang-2), matrix metalloproteinase-3 (MMP-3), and MMP-10, were significantly upregulated. Ang-2 increases vascular permeability by disrupting endothelial morphology and inducing vascular leakiness. MMP-3 and MMP-10, which belong to the same MMP subfamily and share high functional similarity, facilitate Ang-2-induced leakage by degrading the perimatrix, the ECM surrounding the endothelium. These MMP molecules work synergistically with Ang-2 to promote vascular permeability. While both MMP-3 and MMP-10 are used to degrade the endothelial ECM and Ang-2 induces vascular leakiness, both work cooperatively to increase extravasation of cancer cells and exosomes. This extravasation has been shown to significantly contribute to lung metastasis, as inhibiting Ang-2, MMP-3, or MMP-10 reduced lung metastasis. Thus, this indicates that this vascular remodeling pathway is essential for metastatic colonization.

IMMUNE MICROENVIRONMENT CONDITIONING IN THE PRE-METASTATIC NICHE

Remodeling of Myeloid Cells

Myeloid cells are produced within the bone marrow through the differentiation and specialization of hematopoietic stem cells (HSCs). As a tumor matures and becomes malignant, it begins to release a growth factor known as granulocyte colony-stimulating factor (G-CSF). Casbon et al. (2015) reported that G-CSF is upregulated early in tumor progression, and other studies have noted it as a potential indicator of high tumor malignancy (Kawano et al., 2015). After being expressed, G-CSF interacts with HSCs in the bone marrow, causing them to differentiate to form myeloid-derived suppressor cells (MDSCs), which are immunosuppressive. Casbon et al. (2015) also identified that G-CSF targets HSCs early in their differentiation pathway rather than intermediate populations, such as common myeloid progenitors (CMPs). This highlights that primary tumors can increase MDSC numbers by altering hematopoietic differentiation at an early stage, rather than simply converting pre-existing myeloid cells, thereby contributing to an immunosuppressive microenvironment.

After MDSC production, hypoxic primary tumors release a pH-regulatory enzyme, carbonic anhydrase IX (CAIX). This enzyme activates the NF-κB pathway, which causes the primary tumor to secrete more G-CSF (Chafe et al., 2015). Once produced, G-CSF promotes the mobilization of MDSCs from the bone marrow to peripheral organs. Chafe et al. (2015) also demonstrated that this reaction pathway is essential, as G-CSF secretion is dependent on CAIX expression. This shows the dual role of G-CSF as a signaling molecule in both MDSC differentiation and mobilization.

Once MDSCs have reached the target organ, they create an immunosuppressive environment suitable for metastatic growth by inhibiting T-cells and Natural Killer (NK) cells. H. Liu et al. (2023) reported that the primary mechanism by which MDSCs reduce T-cell activation and proliferation is by depleting essential nutrients. MDSCs stimulate the production of indoleamine 2,3-dioxygenase (IDO), which rapidly reduces tryptophan levels within the distant organ. Additionally, MDSCs rapidly consume cysteine without replenishing it in the environment, resulting in lower cysteine concentrations. As both tryptophan and cysteine are essential for T-cell activation and proliferation, their depletion reduces T-cell activity. Additionally, MDSCs secrete nitrogenous species such as nitric oxide (NO), which induces nitration of T-cell receptors and can reduce their responsiveness. This mechanism can also contribute to the inhibition of NK-cell activity, thereby lowering anti-tumor immunity. Finally, MDSCs can promote the differentiation of naive T-cells into regulatory T cells (Tregs), further contributing to immunosuppression.

Remodeling of Neutrophils

Neutrophils are a major type of immune cell and typically act as the first line of defense against bacterial and viral infections in the body. While neutrophils normally contribute to host defense, multiple studies have noted that they can be induced by the primary tumor to become immunosuppressive and facilitate metastatic growth. Two primary papers that show similar mechanisms through which tumors induce immunosuppressive neutrophil phenotypes are Coffelt et al. (2015) and Qi et al. (2022). Coffelt et al. (2015) revealed, in a genetic mouse model, that during tumor progression, the primary tumor releases IL-1β, a pro-inflammatory cytokine involved in immune responses. However, in this instance, IL-1β activates a particular subset of T cells known as γδ T cells, which produce interleukin-17 (IL-17). This promotes the expression of G-CSF, a growth factor discussed above, and causes upregulation of tumor-induced neutrophils, named N2 neutrophils. These neutrophils represent a tumor-associated phenotype that, rather than suppressing metastasis, promotes immune suppression and metastatic growth. These N2 neutrophils promote the expression of inducible nitric oxide synthase (iNOS), which generates nitric oxide to suppress T cells. The study found that the depletion of γδ T cells or neutrophils substantially reduced pulmonary and lymph node metastases in mice, supporting a role for this pathway in PMN formation. Qi et al. (2022) reported a comparable mechanism by which the primary tumor induces N2-like neutrophils and immunosuppression, but through different signaling molecules. Qi et al. (2022) emphasized that particular tumor subtypes, such as triple-negative breast cancer tumors, express high levels of Lin28B, which lowers the amount of a regulatory molecule named let-7. In the presence of Lin28B, Qi et al. (2022) observed a significant upregulation of IL-6 and IL-10, which are known to contribute to N2-like neutrophil polarization. This highlights Lin28B as a tumor-derived regulator of neutrophil reprogramming, as compared to IL-1β in Coffelt et al. (2015). Qi et al. (2022) also found that N2 neutrophils express PD-L2, which can inhibit nearby immune cells, creating an immunosuppressive environment in the PMN. Together, these studies demonstrate that tumors can induce immunosuppressive neutrophil phenotypes through multiple signaling pathways.

Another way in which neutrophils promote metastasis through the PMN is by enhancing the metastatic abilities of tumor cells themselves by secreting factors that promote tumor-cell proliferation. Wculek & Malanchi (2015) found that certain neutrophils produce leukotrienes, pro-inflammatory molecules that can bind to highly metastatic cancer cells, termed metastasis-initiating cells (MICs), and promote their proliferation and increase their proportion within the tumor cell population. N2 neutrophils release leukotrienes, which bind to specific receptors preferentially expressed by MICs, such as BLT1, BLT2, CysLT1, and CysLT2. This promotes MIC proliferation and increases their representation within the tumor population. Although these leukotrienes act on cancer cells already within the lungs, this mechanism is inherently pre-metastatic, as shown by how Wculek and Malanchi isolated neutrophils from pre-metastatic lungs that were already primed for leukotriene production.

Notably, neutrophils not only help build the PMN for metastasis but also play a dual role as cytotoxic agents that kill cancer cells, with this function being dependent on tumor-derived signals. Granot et al. (2011) found that if PMN-resident neutrophils interact with CCL2, secreted by the primary tumor, they

can become tumor-entrained neutrophils (TENs), conditioned by exposure to tumor-derived signals, and can kill cancer cells on contact. The study showed that TENs can generate H2O2 (hydrogen peroxide), which mediates contact-dependent cytotoxicity against cancer cells. Additionally, the study showed that tumors can secrete another signaling molecule, TGF-β, which can inhibit this anti-tumor mechanism. This demonstrates that, in addition to neutrophil population and location, signaling cytokines can determine the functional state of immune cells, thus indicating that the ratio of CCL2 and TGF-β concentrations is able to influence whether neutrophils become TENs.

Remodeling of T-Cells

T-cells are a core leukocyte subtype specialized for a specific antigen and can activate, proliferate, and differentiate into a large number of effector cells upon antigen recognition (Sun et al., 2023). A distinct subset of T-cells is known as regulatory T-cells (Tregs), which have the role of suppressing or regulating the activity of other immune cells, thereby preventing excessive immune responses. However, it has been shown that primary tumors in breast cancer are able to manipulate Tregs to suppress immune cells within the PMN, thereby creating a more immunosuppressive microenvironment that supports metastatic growth. Primary tumors have been shown to increase the population of Tregs available for recruitment and to use pre-existing Tregs within the host body to create an immunosuppressive environment in the PMN.

To induce the conversion of T-cells to Tregs, the primary tumor has been shown to utilize a specialized subset of B2 cells, known as tumor-evoked regulatory B cells (tBregs). Olkhanud et al. (2011) investigated why an extremely high population of Tregs is found in tumor-bearing mice compared to tumor-naive mice, and revealed that the tumor secretome can convert naive B2 cells into tBregs through currently unidentified soluble factors. Importantly, this represents a systemic response rather than a localized one, creating a large population of tBregs in blood and lymphoid organs. After the conversion, these tBregs drive resting T-cells to express the transcription factor FoxP3, which is the distinguishing feature of Tregs. This is achieved through the expression of TGF-β by tBregs, which, along with FoxP3 expression, causes conversion of resting T-cells into Tregs systemically. This pathway can also be linked to metastasis, as the results showed that inhibition of TGF-β expressed by tBregs prevented Treg conversion, and the absence of Tregs significantly impaired breast cancer metastasis to distant organs.

Since systemically available Tregs are required within the distant organ, signals within the PMN are needed to recruit them to the site. Chemokines are signaling molecules that mediate communication between immune cells and are induced within the PMNs to attract and recruit Tregs to the area. In an earlier study, Olkhanud et al. (2009) investigated the relationship between the presence of chemokine receptors and the metastatic success of breast cancer cells. They highlighted that the primary tumor can remotely induce the expression of chemokines CCL17 and CCL22 in lung tissue via its secretome, in the form of soluble and vesicular components. The lung microenvironment contains a large population of natural killer cells (NK cells), which can identify and rapidly destroy virus-infected and tumor cells without needing prior sensitization. This makes them highly effective for maintaining lung tissue as an anti-metastatic environment. To bypass this defense mechanism during metastasis to the lungs, the

primary tumor utilizes the aforementioned chemokines to attract both CTCs and Tregs to the PMN. Olkhanud et al. (2009) demonstrated that the receptor for CCL17 and CCL22, CCR4, is expressed in Tregs and in the specific subset of tumor cells responsible for lung metastasis, but not in NK cells. The use of this receptor allows Tregs to be recruited to the lungs, thereby reducing local immunity and facilitating the recruitment of cancer cells, thereby promoting metastasis. This demonstrates how the primary tumor is capable of inducing specific, favorable signals within the PMN that attract pro-metastatic cells while not directly recruiting anti-metastatic ones. Once a large population of Tregs accumulates within the PMN, they can eliminate NK cells by secreting large amounts of a protein called galectin-1. Interactions between galectin-1 and activated immune cells, such as NK cells, induce cell death via apoptosis (Camby et al., 2006), thereby decreasing NK cell numbers. This weakens an important immune barrier in the distant organ, leading to higher metastasis rates, as evidenced by a substantial increase in susceptibility to metastasis in tumor-bearing mice upon NK cell depletion (Olkhanud et al., 2009). However, the study found that for this mechanism to work, Tregs need a very high population count, as a Treg-to-NK cell ratio less than 4:1 leads to NK cells killing Tregs instead. This may explain why substantial Treg accumulation within the PMN is required for effective suppression of NK cell activity. Together, these findings show that Tregs can accumulate within the PMN via tumor-induced chemokine signaling and suppress NK cell-mediated anti-tumor immunity through galectin-1.

VASCULAR REMODELING AND ANGIOGENESIS IN THE PRE-METASTATIC NICHE

Role of MicroRNAs in Boosting Vascular Permeability and Angiogenesis

Angiogenesis is the formation of new blood vessels from pre-existing vasculature and, while essential for normal growth and repair, is often hijacked by tumors to redirect oxygen and nutrients to promote tumorigenesis. It is also important for the formation and growth of secondary tumors, and its inhibition is an established anticancer strategy that can limit the tumor's nutrient supply (Bielenberg & Zetter, 2015). The most prevalent mechanism investigated in studies of vascular alteration and angiogenesis in PMNs is the use of microRNAs (miRNAs) to reprogram the cell's proteome. miRNAs are short RNA molecules that can be produced intrinsically or transferred between cells. They bind to mRNAs, inhibiting translation and/or promoting mRNA degradation, thereby leading to downregulation of the proteins they encode (Zhou et al., 2014).

Tumors often package miRNAs into exosomes and transport them to human microvascular endothelial cells (HMVECs) in a distant organ, thereby influencing their proteome and, in turn, conditioning the vascular microenvironment. A major miRNA used by breast cancer cells is miR-105, which can augment vascular leakiness. To investigate the mechanisms through which vascular permeability is increased within metastatic sites, Zhou et al. (2014) analyzed and compared metastatic breast cancer lines with non-cancerous lines via deep sequencing, with subsequent validation in mouse models and patient plasma. They identified miR-105 as the primary secretory miRNA associated with increased vascular permeability. Once packaged into exosomes by the primary tumor and delivered to target organs,

particularly the brain and lungs, miR-105 was shown to target HMVECs within the lining of blood vessels and inhibit ZO-1 gene expression. ZO-1 is a primary component of the tight junctions between endothelial cells, which contribute to the barrier between intravascular and extravascular compartments. Inhibiting the production of this protein disrupts endothelial junction integrity and increases vascular permeability. This can facilitate cancer-cell extravasation to distant sites, potentially increasing metastatic success. Zhou et al. (2014) further showed that mice bearing miR-105–expressing tumors developed significantly more metastases than those without miR-105, particularly in the lungs and brain. As discussed in Section 3, both the brain and lungs are characterized by highly selective permeability and unfenestrated capillaries, leading to lower rates of extravasation. The increase in metastases to these organs indicates that CTCs can extravasate into them more readily. Thus, this shows that tumor-derived miR-105 can increase vascular permeability, which may facilitate extravasation and metastatic dissemination.

Beyond permeability, a recently reported and not yet independently replicated mechanism suggests that breast cancer tumors can use miRNAs to increase branching and proliferation within the vasculature, thus inducing angiogenesis within the distant organ. T. Yang et al. (2026) reported that primary tumors use miR-92b-3p-containing exosomes to downregulate phosphatase and tensin homolog (PTEN), a negative regulator of the PI3K/AKT pathway. PI3K/AKT is a broadly expressed signaling pathway that promotes proliferation, migration, and survival, including in endothelial cells (Karar & Maity, 2011). PTEN acts as a regulator of this pathway, limiting excessive signaling and preventing uncontrolled proliferation. The study highlighted that once tumor-derived exosomes containing miR-92b-3p were taken up by HMVECs, they suppressed PTEN activity by downregulating both its mRNA and protein production. Due to reduced PTEN activity, the PI3K/AKT pathway promoted increased endothelial proliferation and migration. Additionally, vessel assays of HMVECs after PTEN downregulation showed that the cells formed ring- and tube-like structures, indicating endothelial tube formation, an in vitro indicator of angiogenic activity. This highlights the primary tumor's ability to promote angiogenesis by removing existing inhibition of host-intrinsic pathways. Additionally, higher exosomal miR-92b-3p levels simultaneously downregulated VE-cadherin, a junction protein similar to ZO-1 that maintains endothelial barrier integrity. This can weaken intercellular junctions and increase vascular permeability, potentially facilitating CTC extravasation. This suggests that miR-92b-3p is utilized as an exosomal miRNA by the primary tumor to promote angiogenesis and increase vascular permeability by inhibiting PTEN/PI3K/AKT signaling and disrupting endothelial junctions. This 2026 study has not yet been independently replicated, and thus these findings should only be treated as preliminary.

Breaching of the Blood-Brain Barrier by Tumor-Derived Exosomes

Beyond the general angiogenic pathways induced across metastatic sites, the tumor also deploys organ-specific mechanisms for tightly regulated barriers such as the brain. The blood–brain barrier (BBB) is a highly restrictive endothelial barrier (Tominaga et al., 2015), limiting the passage of many cells and solutes. In order to reach the brain, tumors must overcome this barrier, and the miRNA mechanism they use here differs from that used in other organs, such as the lungs.

To understand how brain metastasis occurs despite the BBB, Tominaga et al. (2015) created a three-dimensional BBB model and analyzed structural differences before and after the addition of tumor-derived exosomes, in the absence of CTCs. They found that tumors package miR-181c into exosomes, which are taken up by resident endothelial cells and suppress PDPK1, lowering phosphorylated cofilin, a regulator of actin dynamics and cytoskeletal structure. This alters the localization of tight-junction proteins, causing them to move into the cytoplasm rather than remain at the intercellular membrane. This disrupts junctions and creates loosely packed regions in the BBB, thereby increasing permeability and aiding invasion. This mechanism is distinct from the more commonly described role of miRNA in regulating protein expression, as it affects the localization of proteins rather than simply their abundance.

Apart from miRNA, the primary tumor also uses different modes of signaling to induce angiogenesis and permeability, such as through exosomal proteins and long non-coding RNAs (lncRNA). Rodrigues et al. (2019) identified, using ex vivo and mouse models with validation in patient tissue, an exosomal protein CEMIP (cell migration-inducing and hyaluronan-binding protein), found in particularly high concentrations in brain-tropic exosomes. Once taken up by HMVECs and microglial cells, it upregulated various signaling molecules, such as Ptgs2, Tnf, and Ccl/Cxcl family chemokines, causing a pro-metastatic and inflammatory response that increased vascular branching in terms of both vessel length and branch number. This may increase metastatic potential by increasing the surface area for invasion and a more permissive microenvironment for secondary tumor growth and proliferation. This is supported by the fact that the metastatic success rate was 4-fold higher in brain tissue preconditioned with brain-tropic exosomes than in non-preconditioned brain tissue.

Another set of key molecules used by breast cancer tumors to increase permeability in the brain are long, non-coding RNAs (lncRNAs). lncRNAs are much longer (>200 nucleotides) compared to miRNAs (18-24 nucleotides), and can interact with DNA, RNA, and proteins rather than exclusively interacting with mRNA (Ratti et al., 2020). To investigate the relation between lncRNAs and brain metastasis, Lu et al. (2020) examined brain-tropic exosomes introduced to a three-dimensional BBB model, similar to Tominaga et al. (2015), and identified the use of exosomal lncRNA GS1-600G8.5 to disrupt BBB integrity. This confirmed the use of RNA species other than miRNAs in forming the PMN. Notably, this was demonstrated using an in vitro BBB model only, with in vivo validation yet to be performed. GS1-600G8.5 caused loss of expression of tight junction proteins ZO-1 and Claudin-5, which have a key role in maintaining the integrity of the BBB by forming intercellular junctions between endothelial cells. This led to increased vascular leakiness, which may facilitate the passage of exosomes and other tumor-derived factors across the BBB. It is important to note that this effect was not dependent on direct exposure to tumor cells, as induction of vascular leakiness enabled poorly metastatic tumor cells to also cross the BBB and invade brain tissue. Thus, apart from miRNA, breast cancer tumors can use multiple modes of signaling, including exosomal proteins and lncRNA, to disrupt key components of BBB stability and promote a more permissive environment for brain metastasis.

CONCLUSION

The many mechanisms through which PMN formation contributes to metastasis in target organs make metastatic disease difficult to prevent and treat. This review presented and analyzed the mechanisms by which tumor-derived secreted factors contribute to PMN formation, such as through organ-specific exosome composition, stromal remodeling, suppression of the local immune response, and induction of angiogenesis and vasculature changes in resident capillaries. This highlights that PMN formation does not proceed in a single, definitive way, but instead involves coordination between multiple systems within the target organ to create a microenvironment that is permissive to metastasis.

A recurring theme noticed through all the mechanisms is redundancy, where multiple molecular interactions converge to produce similar functional outcomes. For example, the signaling pathways of three separate molecules, miR-105, miR-181c, and lncRNA GS1-600G8.5, can ultimately disrupt tight-junction integrity. This indicates that similar responses can be induced through multiple molecular pathways, demonstrating the complexity and redundancy of PMN formation.

These findings also point towards two real-world implications. While extensive research is currently being done on PMN formation, this review also highlights areas where further research is needed. Exosomes are present throughout the circulation and contain molecular cargo that may contribute to organ-specific PMN formation by the primary tumor before metastasis occurs. This highlights the potential importance of liquid biopsy and analysis of exosomal contents to detect proteins, cytokines, and genetic material that may indicate organ-specific PMN formation and metastatic risk. Additionally, the redundancy of signaling pathways suggests the potential need for combination therapeutic approaches, as targeting a single molecular pathway may not be sufficient to prevent PMN formation when alternative pathways can produce similar effects.

Several limitations affect these conclusions. Across these areas, particularly in angiogenesis, there remains limited research on which specific exosomal contents drive particular molecular pathways, with many studies focusing on the most readily identified cargo, such as miRNAs, rather than proteins and other mediators. Thus, it is important to continue research in these fields, with a targeted focus on specific pathways and mechanisms that remain incompletely understood. In addition, a significant portion of the evidence base reviewed here is preclinical. Many mechanisms were established using mouse models, xenografts, or in vitro systems, with human data largely limited to correlative observations in patient samples. Therefore, clinical translation of exosomal cargo as a biomarker remains prospective rather than established.

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