The cancer cell secretome drives cooperative manipulation of the tumour microenvironment to accelerate tumourigenesis

Shona Ritchie 1,2  Daniel A. Reed 1,2  Brooke A. Pereira 1,2*  Paul Timpson 1,2*

1 The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, New South Wales 2010, Australia
2 St. Vincent’s Clinical School, Faculty of Medicine, University of New South Wales, Sydney, New South Wales 2010, Australia

Abstract

Cellular secretions are a fundamental aspect of cell–cell and cell–matrix interactions in vivo. In malignancy, cancer cells have an aberrant secretome compared to their non-malignant counterparts, termed the “cancer cell secretome”. The cancer cell secretome can influence every stage of the tumourigenic cascade. At the primary site, cancer cells can secrete a multitude of factors that facilitate invasion into surrounding tissue, allowing interaction with the local tumour microenvironment (TME), driving tumour development and progression. In more advanced disease, the cancer cell secretome can be involved in extravasation and metastasis, including metastatic organotropism, pre-metastatic niche (PMN) preparation, and metastatic outgrowth. In this review, we will explore the latest advances in the field of cancer cell secretions, including its dynamic and complex role in activating the TME and potentiating invasion and metastasis, with comments on how these secretions may also promote therapy resistance.

Keywords

Cancer cell secretome, Tumour microenvironment, Stroma, Pre-metastatic niche

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2. Monica Neagu, Immunology Department, “Victor Babes” National Institute of Pathology, Bucharest 050096, Romania
   Department of Pathology, Colentina University Hospital, Bucharest 020125, Romania
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3. Anil K. Sood, Department of Gynecologic Oncology & Reproductive Medicine, and Center for RNA Interference and Non-Coding RNA, The University of Texas MD Anderson Cancer Center, Houston, TX, 77030, USA
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**Corresponding authors:** Brooke A. Pereira (b.pereira@garvan.org.au); Paul Timpson (p.timpson@garvan.org.au)

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The cancer cell secretome

The term “secretome” can be defined as any factor (soluble or insoluble) that is released or secreted into the extracellular space. The secretome can consist of a diverse array of factors including chemokines, cytokines, growth factors, coagulation factors, hormones, enzymes, glycoproteins, and microRNAs (miRNAs). These factors can be secreted individually or contained in vesicles such as extracellular vesicles (EVs) (e.g., exosomes) or nanovesicles (e.g., exomeres). In normal tissues, the secretome is tightly regulated to maintain tissue homeostasis, with secreted proteins and their cognate receptors functioning as the main mechanism by which cells and tissues communicate. The cell secretome is diverse in its function, where secreted proteins can act in an autocrine, paracrine, or endocrine manner, both locally and systemically. Soluble proteins are synthesised as precursors at the endoplasmic reticulum (ER) and then transported to the Golgi apparatus, where they are packaged and excreted. This “classical” secretory process is completed when secretory vesicles fuse with the plasma membrane and their contents are expelled from the cell surface into the microenvironment. Several other “non-classical” pathways of secretion have also been described, where synthesised proteins may be secreted directly from the ER in a Golgi-independent manner. Exosomes, for example, are composed of intraluminal vesicles inside multivesicular bodies which fuse with the plasma membrane independent of the Golgi.

Recent revision of the human secretome has found that approximately 13% of all human protein-coding genes code for secreted proteins. However, for some secretory organs such as the pancreas and salivary gland, secreted proteins account for the majority of transcriptional outputs, as expected. In cancer, the secretome is significantly altered, often displaying widespread changes across multiple cellular processes. A recent pan-cancer analysis conducted by Robinson et al. established that the cancer cell secretome is markedly distinct from paired healthy tissues. In this analysis, secretome transcriptomic signatures of 32 cancer types were compared with 30 healthy counterparts, specifically comparing genes that were expected to be most differentially expressed between different cancer types and healthy participants. Interestingly, the subset of the secretome that exhibited the strongest differential expression across the majority of the cancer types included loss of tumour suppressors (putative or established) as well as loss of genes involved in cell–cell/matrix adhesions and the immune response. Strikingly, when assessing for transcripts which were overexpressed across the cancer cohort, most top-ranking genes were related to extracellular matrix (ECM) structure, composition, and modification as well as vascular remodelling, although there were less shared transcript commonalities between different cancers for this analysis. Overall, this systematic study highlights the dynamic, complex nature of the cancer cell secretome and its role in the pathophysiology of cancer (Figure 1). It suggests that there is a common global shift towards pro-tumourigenic pathways, including ECM and vascular remodelling and loss of tumour suppressors; however, it is important not to overlook other secretome pathways in specific cancers. Large-scale studies such as these are interesting to gain a global perspective, but care should be taken not to over-speculate, so as not to miss critical cancer type-specific nuanced factors that can drive progression.

The cancer cell secretome activates the tumour microenvironment

Basement membrane degradation

During early malignancy, reciprocal heterotypic paracrine signalling between tumour cells and other tumour microenvironment (TME) components triggers a cascade of biochemical and biomechanical changes, creating a dynamic niche of cell–cell and cell–matrix interactions. For many solid cancers, a prerequisite for the initiation of malignancy involves the secretion of ECM remodelling enzymes by newly transformed tumour cells to degrade the basement membrane (BM) (Figure 2). The metalloproteinase families are the main enzymes within the secretome that carry out ECM degradation. Two branches exist: matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAM/Ts), both of which are frequently found to be overactivated in cancer. MMPs are zinc-binding endopeptidases with varying targets within the ECM. For example, MMP1 digests collagen III, MMP3 and MMP10 prefer fibronectin and laminins, and MMP2 and MMP9 break down gelatine.

Overall, and without targeted inhibition, MMPs, ADAMs, and
Figure 1. The cancer cell secretome drives a pro-tumourigenic environment. During the process of cancer development, the secretome is markedly changed compared to healthy tissue, with increased levels of secretion resulting in a change to many key processes enhancing tumour growth. Examples of pathways affected by tumour cell-derived secretion include an increase in fibroblast activation, extracellular matrix (ECM) deposition, and vascular remodelling while key pathways in tumour suppression, cell–cell matrix adhesion, and regulation of the immune system are lost.

Figure 2. The cancer cell secretome drives tumour microenvironmental changes enhancing tumour growth, invasion, and metastasis. Tumour cell secretion activates a number of pro-tumourigenic processes. These events include basement membrane degradation, activation of cancer-associated fibroblasts (CAFs), deposition of extracellular matrix (ECM), angiogenesis at the tumour site, and immunomodulation to favour an immunosuppressive environment. Examples of key tumour-secreted proteins that affect each major process are shown. ADAM/T, a disintegrin and metalloproteinase/with thrombospondin motif; CCL, C-C motif chemokine ligand; CSF-1, colony-stimulating factor 1; CXCL, C-X-C motif chemokine ligand; IFN, interferon; IGF2, insulin-like growth factor 2; IL, interleukin; MDSC, myeloid-derived suppressor cell; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; SDF1, stromal cell-derived factor 1; TAM, tumour-associated macrophage; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumour necrosis factor; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.
TIMPs work collectively to cleave and degrade ECM molecules, which allows for invasion of tumour cells beyond the BM. MMP family members have also been shown to participate in other cancer-promoting actions like mediating communication between the tumour and stroma, allowing for paracrine signalling to recruit and activate fibroblasts, vascular cells, and immune cells. Activated stromal cells then signal back to the tumour cells and the TME, resulting in a complex, pro-tumourigenic loop (Figure 2).

**The cancer secretome influences angiogenesis**

In cancer development, newly formed tumours will initially utilise pre-existing vasculature to proliferate and expand. However, soon cancer cells will begin to recruit endothelial progenitors and supporting pericytes to the TME via secreted factors to grow and remodel new vasculature, termed angiogenesis (Figure 2). Cancer cell hypoxia has become a well-established phenomenon for inducing angiogenesis, where hypoxia-driven pH changes to the TME can result in the recruitment of vascular cells by cancer cell-secreted proteins such as vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), stromal cell-derived factor 1 (SDF-1/CXCL12), and angiopoietins as well as genetic material such as miRNAs (Figure 2). Mounting evidence is showing that many of these factors are secreted from cancer cells in EVs and that the acidic TME might encourage the production, release, and survival of EVs to encourage angiogenic growth.

Intriguingly, several EV-packaged miRNAs have been described in different cancers, where they are received by endothelial cells to promote the proliferation of new vessel growth and therefore migration. Hsu and colleagues described this process in hypoxic lung cancer cells, where they showed increased exosomal secretion in hypoxic compared to normoxic conditions. Within the exosomes, upregulated miR-23 expression targeted hypoxia-inducible factor 1-alpha (HIF1α) to promote pro-angiogenic activities of endothelial cells. In glioblastoma, hypoxic cancer cells upregulated the secretion of miR-182-5p, stimulating a potent accumulation of VEGF receptor (VEGFR) and repression of tight junction molecules. This combination resulted in enhanced angiogenesis and increased permeability of further exosomes. Tumour cell-derived exosomes may also target other stromal cells to encourage the secretion of pro-angiogenic factors. Breast cancer exosomes targeted adipocyte-derived mesenchymal stem cells to transform them into a myofibroblast-like phenotype, resulting in the increased secretion of VEGF, SDF-1, and TGF-β.

This cancer cell-derived exosomal paracrine pathway resulted in the upregulation of angiogenic pathways, with myofibroblasts acting as the intermediate player. Strikingly, Follain and colleagues have highlighted that extravasation and endothelial remodelling is partly blood flow dependent and leads to increased metastases. Their studies revealed that the vascular endothelium wall was actively remodelled around the extravasating circulating tumour cell (CTC), and hemodynamic cues from the sheer force of blood flow activated VEGFR pathways to encourage the exit of the cancer cell towards a metastatic site. Thus, although this review focuses on the cancer secretome, the work by Follain et al. is one of many studies that showcases the importance of other dynamic physiological cues that also contribute towards developing a cancer-permissive environment. As well as this, angiogenesis allows increasing numbers of immune cells to infiltrate and modulate the immunosurveillance landscape through increased permeability. (Figure 2). This reiterates the complex cancer paracrine pathways that utilise the cancer cell-activated microenvironment. Overall, secretions from cancer cells dictate the initiation and maintenance of pro-angiogenic pathways that allow for tumour growth during altered physiological conditions. These new vessels also provide an increased likelihood for primary tumour cells to migrate towards the systemic vasculature, where they may begin their journey towards a secondary site.

**Cancer cell secretome and immunomodulation**

The cancer cell secretome is critical to promoting immunosuppression in the TME. Immunosuppression can occur when cytotoxic T cells are impeded by other immune cell populations, such as tumour-associated macrophages (TAMs), regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs), or are exhausted because of prolonged cancer cell antigen presentation (Figure 2). The roles of immune cells can also be dictated by cancer cells throughout tumour progression. TAMs, for example, are in a state of potential flux and can be primed to switch between an M1/M2 state by the cancer secretome. In simplified terms, TAMs in a pro-inflammatory M1-like state can be recruited to the tumour site by cytokines such as interleukin 12 (IL-12), tumour necrosis factor (TNF), and interferon gamma (IFNγ) during early oncogenesis. Initially, TAMs in this state are believed to be anti-tumourigenic, releasing cytotoxic agents that damage cancer cells such as nitric oxide (NO), and can also destroy malignant cells by engulfing them. However, prolonged TAM activity can eventually cause chronic inflammation and genomic instability in neoplastic cells, promoting malignant proliferation and progression. Additionally, cancer cells can repolarise TAMs towards an M2 state through secretion of metabolism re-programming factors, such as colony-stimulating factor 1 (CSF-1), and metabolites, such as lactate. In these conditions, TAMs are then able to secrete pro-tumourigenic factors that can further modulate the TME such as VEGF (pro-angiogenic), IL-10 (immunosuppressive), EGF (growth promoting), and MMPs (matrix remodelling) (Figure 2). Importantly, a complex mosaic of TAMs in M1/M2 states can occur spatially and temporally in any given tumour, which therefore promotes different conditions of inflammation and immune surveillance within the same tumour area. Further interrogation of fluctuating TAM polarisation should therefore be considered for future drug targets. Neutrophils can also be activated by the cancer cell-derived secretions, with a recent study by Teijeira et al. reporting that cancer cell-derived chemokines (IL-8, CXCL1, CXCL2, CXCL8) activate the CXCR1 and CXCR2 receptors on neutrophils, resulting in the formation of neutrophil extracellular traps (NETs). NETs act to physically shield cancer cells from the immune system, in particular cytotoxic T cells and NK cells, promoting cancer growth. NETs have also been implicated in stimulating dormant cancer cells, pre-metastatic niche (PMN) formation, and alteration of mitochondrial activity. Furthermore, inhibition
of neutrophil-upregulated CXCR2 expression improved response to checkpoint inhibitors, which slowed tumourigenesis, suppressed metastasis, and improved infiltration of cytotoxic T cells in pancreatic cancer64. This suggests that neutrophils have diverse tumour-promoting functions and have therapeutic targeting potential.

MDSCs also play a significant role in promoting immune-suppression in the TME. MDSCs are recruited from the bone marrow to the tumour site via cancer cell-derived chemokines such as CCL2, CCL5, CXCL5, and IL-865. Here, they initiate several immunosuppressive processes, which impact other immune cells in their surroundings, such as nutrient deprivation of T cells. MDSCs can also cause inhibition of lymphocyte homing, where the production of damaging molecules such as reactive oxygen species (ROS) and NO inhibit the expression of immune checkpoint molecules66. As well as during cancer progression, MDSCs have been utilised these tactics to promote cancer relapse during chemotherapy67. Rong and colleagues showed that doxorubicin (Dox)-resistant breast cancer cells secrete prostaglandin E2 (PGE2) to support the expansion of MDSCs68. This results in inhibition of CD4+CD25 T cells and enhanced immune chemotherapy resistance67. MDSCs also interact with cells of the adaptive immune system such as Tregs to impede immunosurveillance. One study has shown that MDSCs must first be activated in the TME to permit the differentiation and infiltration of Tregs69. However, more recently, Lee et al. provided evidence that Tregs can modulate MDSC expansion and function through TGF-β65. Tregs suppress the inflammatory response and control anti-cancer immunity and are identified by the expression of the master transcription factor forkhead box protein p3 (FOXP3). They remain a difficult subset of T cells to target owing to commonality with cytotoxic T cells, which are generally protective70. As well as paracrine recruitment via other immune cells, cancer cells can directly recruit Tregs to initiate anti-cancer immunity. For example, in pancreatic ductal adenocarcinoma (PDAC), Wang et al. found that FOXP3-positive cancer cells secreted CCL5 to recruit Tregs into the TME, which can be blocked to repress Treg influx and tumour growth71. Thus, cancer-promoting secretions activate several interactive immune pathways to ultimately shield the invading tumour cells from a functional immune response.

Cancer cell secretome activates cancer-associated fibroblasts and alters the extracellular matrix

Cancer cell-derived secretions also recruit mesenchymal stem cells (MSCs) and fibroblasts, which become activated to form heterogeneous populations of cancer-associated fibroblasts (CAFs) capable of distinct cancer-promoting functions72–77 (Figure 2). CAFs are the most abundant cell type in the tumour stroma and have far-reaching effects in the TME, where they can act to both restrain78–81 and promote tumour development and progression72,75,82,83. CAFs can also indirectly influence tumour progression through the regulation of metabolic reprogramming, angiogenesis, and inflammation in the TME65–67 (Figure 2). Crucially, CAFs are the main producers of structural ECM components such as fibrillar collagens, fibronectin, and hyaluronic acid84,85. ECM is commonly dysregulated across a number of solid malignancies86. ECM molecules accumulate as CAFs proliferate in early cancer development and contribute to an altered ECM composition, porosity, and topography as well as increases in tissue stiffness88,90,92. Thus, cancer matrix remodelling consists of a juxtaposition of processes: stromal cell recruitment and proliferation leads to excessive deposition of large ECM components such as collagens and proteoglycans, while the simultaneous breakdown of ECM elements from cancer cell-derived secretory factors allows for BM degradation and the process of invasion to be initiated. ECM provides both biochemical and biomechanical cues to promote cancer93–95. The ECM can directly influence tumour cells but also indirectly promote angiogenesis, inflammation, and further stromal activation73,76,93,96. ECM is also implicated in the preparation of the metastatic niche and metastatic outgrowth, which will be discussed later in this review. Interestingly, Oudin and colleagues found that cancer-driven ECM remodelling promotes haptotaxis (gradient-directed motility) of cancer cells up a fibronectin gradient and towards the bloodstream97. The study found that the integrin αβ1 interacts with an isoform of an actin-regulating protein, MENAβ5, which allows the cells to move towards an increased concentration of fibronectin closer to the perivascular compartment97. Although this mechanism is tumour cell intrinsic, it manipulates the ECM in such a way that it provides a remodelled path for the cancer cells to travel through, thereby encouraging metastasis.

Aberrant ECM in malignancy is generally thought to be secreted by stromal cells such as CAFs. This was partially disputed by Tian et al., who assessed the contribution of ECM deposition from stromal cells and epithelial cancer cells in pancreatic ductal adenocarcinoma (PDAC) using a cross-species identification approach98. Here, the authors report that over 90% of tumour-associated ECM is produced by stromal cells, with the other 10% being tumour cell-derived ECM proteins98. The authors implied that proteins originating from CAFs were associated with both anti- and pro-tumourigenic elements, whereas cancer cell-derived ECM were associated with pro-tumourigenic actions more frequently98. Although this is an interesting concept that should be explored further, it is important to note that this work was performed with transplanted human PDAC tumours grown in a murine host, where they identified the source of ECM by species, thereby appointing tumour-derived ECM from humans and all stromal-derived ECM as mouse. Care should be taken to not solely attribute pro-tumourigenic ECM characteristics to only one compartment, i.e. the cancer cells, as CAFs also feedback to cancer cells to promote tumour progression and development. Overall, the reciprocal heterotypic communication between tumour and stromal cells allows continual synthesis, production, and secretion of an abundance of transformative proteins, which ultimately aids tumour invasion and metastasis. The studies discussed
have underscored the complexity of tumour–TME crosstalk, and the need for a better understanding of the dynamic environment, in order to improve the efficacy of anti-stromal therapies in desmoplastic cancers.

**The cancer cell secretome promotes preparation of the pre-metastatic niche, metastatic migration, and outgrowth**

The process of metastasis involves many levels of evasion by cancer cells and their associated secreted factors. Survival in the systemic vasculature is relatively unlikely for cancer cells, and successful growth at secondary sites relies on malignant cells to dynamically shape the new “ecosystem” before and during expansion. Metastatic niches can either be established as CTCs arrive at a specific metastatic site or prepared in advance by primary or CTC-secreted factors, resulting in the development of the PMN. The precise secretome and molecular mechanisms of the PMN vary greatly between cancer types but, in general, can be broken down into a series of steps, beginning with BM breakdown, alteration of resident cells in the target organ, remodelling of the PMN ECM, recruitment of non-resident cells such as bone marrow-derived haematopoietic progenitor cells and widespread systemic recruitment of immune cells. Cancer cells can also educate each other to become more migratory and pro-metastatic. For example, Zomer et al. reported that malignant tumour cells can trigger migratory behaviour and metastatic capacity in tumour cells which were less malignant by short- and long-range exchange of EVs in vivo. A later study by the same group established that the highly malignant EVs contain both RNA and proteins enriched for migratory behaviour. This is further supported by a study by Gangoda et al., who reported that cells with varying metastastic potential had differential exosomal cargo, which could then facilitate metastatic outgrowth at distinct sites. Similarly, Kalra et al. showed that EVs can transfer mutant β-catenin to the recipient cells, promoting cancer progression via activation of Wnt signalling. Several eloquent reviews discuss the influence of the cancer secretome at each step in detail. This review will highlight the most recent findings regarding the tumour secretome in organotropism, PMN preparation, and metastatic maturity.

**Organotropism in metastasis**

Common sites of metastasis can include the lymph node, bone, liver, lung, brain, and peritoneum. Depending on the origin of the primary tumour, specific organs are more prone to PMN transformation and primary metastatic maturity (Figure 3a). Recent work by Hoshino et al. showed that exosomes released by metastatic cancer cells preferentially precondition resident cells at their PMN organ choice in an integrin-dependent manner. Remarkably, they also showed that breast cancer cell-derived exosomes (that colonise to the lung) could redirect the disseminating cancer cells of a different type from its usual PMN choice of bone and instead metastasise to the lung. They suggested this behavioural change may be down to instructive integrins expressed on the exosomes which could direct the migration of cancer cells. For example, integrin αvβ3, directed cells to the liver, whereas αvβ3 encouraged migration to the lung. This was also shown in pancreatic cancer by Costa-Silva et al., who observed that pancreatic cancer exosomes can direct PMN establishment to the Kupffer cells of the liver, and is discussed more later in the review. Therefore, although these concepts require further study, it showcases novel potential mechanisms for cancer cell-directed organotropism in metastasis.

Epithelial-derived cancers often initially extravasate into the lymphatic system (Figure 3b), where CTCs encounter immune cells that are programmed to modulate future immune responses, as extravasation from the primary tumour continues. Lymph node metastasis predicts poor patient outcomes in several cancers including breast, prostate, lung, melanoma, and colorectal cancers, as it signifies high probability that malignancy will spread to a number of organs from lymphatic circulation. Recently, Ubellaeker and colleagues revealed research to explain why it is common for cancer cells, as in melanoma, to first metastasise to the lymph rather than directly into the bloodstream. As previously mentioned, after cancer cells extravasate into the systemic vasculature (Figure 3c), most of them die owing to the extreme change of environment, where they battle with loss of ECM adhesion, shear stress from blood flow, and impacting red blood cells. Additionally, ROS molecules are mediators of cancer cell death within the circulation. Ubellaeker et al. showed that tumour cells circulating the systemic vasculature are killed by a form of ROS, known as ferroptosis, which is an iron-dependent death pathway that results in lipid peroxidation. Therefore, to avoid this, metastatic melanoma cells were suggested to first congregate at the lymph nodes as a survival mechanism. This elegant study by Ubellaeker et al. demonstrated that melanoma cells that first move through lymph followed by circulatory exit are more likely to survive than melanoma cells that enter the bloodstream directly. At lymphatic metastases, other studies have also shown that cancer cells successfully move into the circulation via perfusion. Before the arrival of CTCs in lymph nodes, however, the tissue has been primed by secretions from primary tumour cells. For example, tumour cell-secreted IL-6 from triple negative breast cancer (TNBC) cells triggers STAT3 phosphorylation in lymphatic endothelial cells. This leads to a cascade of transcriptional events that results in CCL5 expression in the lymphatic vasculature, allowing for the recruitment and infiltration of tumour cells.
Furthermore, the previously mentioned study by Costa-Silva and colleagues reported that Kupffer cells in the liver selectively uptake malignant pancreatic exosomes containing pro-fibrotic signalling molecules (TGF-β and fibronectin), supporting liver-specific PMN formation in PDAC\(^{108}\). Specifically, they showed that exosomal macrophage migration inhibitory factor (MIF) induced these changes in the liver and that this was conserved in human PDAC cases (Figure 3d)\(^{116}\).

Bone is often another common target of PMN from primary tumours and their secreted factors. Cascades targeting osteocytes can activate two pathways of malignancy: either osteoblastic (bone-forming) or osteolytic (bone-degrading) metastasis. Lysyl oxidase (LOX) secretion has been implicated in breast cancer, where under hypoxic conditions the ECM-modifying enzyme promotes osteolytic lesions\(^{118-120}\). Other ECM remodelling enzymes, such as MMPs derived from prostate cancer cells, are also involved in bone metastasis by activating osteoblast differentiation \(\text{via}\) NFκB signalling (Figure 3d)\(^{121}\). Strikingly, nutrient availability can determine the level of ECM remodelling that occurs at metastatic sites. Elia \(\text{et al.}\) found that breast cancer cells require the nutrient pyruvate to cause ECM remodelling \(\text{via}\)
collagen hydroxylation at metastatic lung sites\textsuperscript{122}. When pyruvate metabolism was inhibited in pre-clinical models of breast cancer, lung metastasis was significantly impaired\textsuperscript{122}.

In melanoma, breast and lung cancer, the brain is a favoured organ of metastasis\textsuperscript{123}; however, these cancers can also metastasise to other organs. Distinct from all other organs, the blood–brain barrier (BBB) offers an additional layer of protection from CTCs and immune cells; therefore, establishment of the PMN of the brain requires the breakdown of this barrier by secreted factors. Molecular mechanisms occurring at this boundary have previously had limited clarity due to lack of available human samples. Recently, Klotz and colleagues identified an interaction between luminal breast cancer-secreted semaphorin (SEMA4D) and Plexin-B1 on human brain microvascular cells, which positively transmigrated CTCs through the BBB\textsuperscript{124}. Interestingly, SEMA4D has also been involved in the formation of bone metastases, suggesting a common PMN regulator of this aberrantly secreted semaphorin transmembrane protein\textsuperscript{125}. Overall, metastatic organotropism is yet another complex process often governed by cancer cell secretions, influencing both their immediate and their distant microenvironment. As highlighted, pathways initiating the choice of PMN are cancer type dependent and in some cases can override other cancer types to coerce an atypical secondary site\textsuperscript{108}. This suggests a varying metastatic degree exists between cancer cell types, and the transfer of metastatic potential between cancer cells could be an interesting topic to consider in the future.

**Vasculature remodelling at the pre-metastatic niche**

Another crucial factor of PMN formation is the remodelling of vasculature at the PMN organ via primary tumour-secreted factors. Altered vascularisation of the PMN means permeability is aberrantly increased, allowing enhanced penetration of circulating pro-tumourigenic factors into the target organ (Figure 3c). This pathophysiological reorganisation has been associated with increased metastatic burden in several cancers\textsuperscript{126,127}. VEGF is a well-characterised tumour-derived secretory factor involved in the endothelial reorganisation of the PMN through the activation of BMDCs and a multitude of signalling pathways\textsuperscript{106,128}. More recent work has established that downstream targets of VEGF become compromised, which promotes tight junction disruption and hyperpermeability observed in PMN\textsuperscript{129,130}. Occludin, a transmembrane protein that regulates tight junctions, was found to be downregulated in the pre-metastatic lung from a metastatic breast cancer murine model\textsuperscript{120,130}. This was suggested to occur from cancer cell-derived VEGF-stimulated phosphorylation and ubiquitination of occludin, therefore impacting the tight junction-regulating function of occludin. Another recent study has suggested that vascular leakiness as well as angiogenesis in the PMN can be observed prior to the arrival of CTCs\textsuperscript{131}. Although this is not currently considered a step in the “metastatic cascade”, the authors present the idea that metastasis can occur very rapidly without a transient stage of dormancy and therefore PMN angiogenesis should be considered as a crucial player in metastatic success\textsuperscript{131}. Using an orthotopic model of metastatic breast cancer, the authors showed accumulation of MDSCs in the lungs during PMN formation but before cancer cell arrival\textsuperscript{131}. This has been previously established\textsuperscript{132,133}; however, this study shows that the recruitment of MDSCs by cancer cell-derived IL-\textsuperscript{10} and TNF secretion could aid in PMN angiogenesis by revealing a number of pro-angiogenic factors secreted by MDSCs, including MMP-\textsuperscript{9}\textsuperscript{135}. Furthermore, they showed this recruitment of MDSCs is regulated by a complement-dependent pathway, complement C5a receptor 1–MDSC (C5aR1–MDSC) axis, and, when pharmacologically blocked, pro-angiogenic factors were reduced, vascular density diminished, anti-tumour immunity improved, and ultimately metastatic burden was lessened\textsuperscript{133}. This is especially exciting, as C5aR1 blockade can be combined with currently used immunotherapies such as *Listeria monocytogenes*-based vaccines, which aim to stimulate T cell responses to tumour and metastatic vasculature formation\textsuperscript{134,135}. In combination, efficacy was improved and showed more success in prohibiting metastases than the standard-of-care drug sunitinib, a pan-inhibitor of VEGFR\textsuperscript{135}. Studies such as this exemplify the benefit of stepping outside of the previously established PMN paradigm, offering hope for future alternative cancer therapies as a result.

**ECM remodelling at the pre-metastatic niche**

As well as the altered vasculature, the ECM undergoes extensive remodelling at the PMN to house invading and expanding cancer cells. This occurs in two processes and is directed by the primary tumour: the depositing of new ECM components and the degradation of the pre-existing ECM. Activated fibroblasts and MDSCs play a large role in establishing the cancer-permissive landscape (Figure 3d). A recent study found that the stress-induced p38 protein kinase (encoded by *Mapk14*) is activated in lung fibroblasts by metastatic malignant cell secretory factors\textsuperscript{136}. Activation of lung fibroblasts caused a cascade of pro-metastatic downstream effects, including repression of IFN\textsuperscript{α/β} receptor subunit 1, which has previously been shown to be inhibitory towards PMN formation in metastatic melanoma models\textsuperscript{137}. Fibroblast activation protein (FAP) expression was also induced in the activated fibroblasts, resulting in excessive fibronectin deposition and neutrophil recruitment via cancer cell-secreted CXCL1\textsuperscript{138}. Preparation of the PMN ECM has implicated more than the cancer cell secretome, and recent evidence shows that CAFs within the primary tumour stroma are able to release factors that induce fibroblasts in the distant PMN of the lung\textsuperscript{138}. CAF-derived EVs were detected by lung fibroblasts in the PMN, mediated by integrin α\textsubscript{v}β\textsubscript{3}\textsuperscript{138}. This triggered TGF-β\textsubscript{1} signalling pathways in lung fibroblasts and prompted extensive remodelling of lung ECM. EVs released by CAFs were shown to be more influential, in this case, in remodelling the PMN ECM over EVs released by cancer cells\textsuperscript{138}. These studies offer a new perspective for ECM remodelling of the PMN, and shows that the cancer cell is capable of manipulating the systemic environment for tumour growth advantage on many levels. Perhaps this is indicative of a mesenchymal-common response reverberating through the systemic cancer-associated secretome, which is a powerful tool to harness for the future.

**Future perspectives**

Taken together, these studies and others have shown that cancer cell-derived secretions are implicated in all steps of disease induction, development, and progression. However, many
mechanisms through which cancer achieves these processes are still being elucidated. Through increased availability of pre-clinical and patient samples, recent research has highlighted just how complex and intertwined cancer pathways can become when interacting with the tumour stroma and the PMN. Although it was only briefly discussed in this review, many other factors in the TME can dictate the progression of disease. For example, the influence of heterogenous subpopulations of CAFs recently described by Ohlund et al.163 and others164,165 have been shown to have diverse roles in cancer progression and therapy resistance. Moreover, biomechanical cues from collagen reorganisation in the desmoplastic stroma can largely impact proliferation and migration.166 These stromal aberrations are first activated by the cancer cell, but the dynamic crosstalk between the neoplastic cell and the stromal environment has a monumental impact on cancer progression. These supportive cells are just as essential for the disease to proceed, and new discoveries between cancer and matrix are frequently being revealed.

Furthermore, the intratumoural heterogeneity of patient tumours poses another challenge in the treatment of cancer. Genetically engineered mouse models are essential for biomedical research, yet they often harbour specific global mutations that do not fully align with the genotype of patient tumours. New evidence is attempting to “map” a more realistic version that do not fully align with the genotype of patient tumours. By doing so, it will expand and connect the network of different cancer research areas, allowing for multidisciplinary and novel discoveries in the future of medical health.

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