Role of the Hypoxic-Secretome in Seed and Soil Metastatic Preparation

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Simple Summary: During tumor growth the aberrant or absent vasculature causes hypoxia, an important decrease in the supply of oxygen to the cells that causes an adaptative response in the microenvironment of the tumor. Hypoxia activates the expression of genes that control several essential cellular processes. Among others, hypoxia induces the expansion of cancer stem cell (CSC) pools and promotes the transcription and secretion of factors that will adapt niches in different organs to receive, proliferate and promote the survival of malignant cells from the primary tumor, forming metastasis. CSCs at the secondary site also interact with stromal cells in the secondary organ to promote metastasis. Several cytokines released by cells within the secondary tumor microenvironment determine the tissue inflammatory infiltration and the cancer-associated phenotype of the immune component. Therefore, hypoxia initiates a cascade of physiological responses that not only affect the primary tumor, but also prepare secondary niches in distant organs for the apparition and development of metastasis.

Abstract: During tumor growth, the delivery of oxygen to cells is impaired due to aberrant or absent vasculature. This causes an adaptative response that activates the expression of genes that control several essential processes, such as glycolysis, neovascularization, immune suppression, and the cancer stemness phenotype, leading to increased metastasis and resistance to therapy. Hypoxic tumor cells also respond to an altered hypoxic microenvironment by secreting vesicles, factors, cytokines and nucleic acids that modify not only the immediate microenvironment but also organs at distant sites, allowing or facilitating the attachment and growth of tumor cells and contributing to metastasis. Hypoxia induces the release of molecules of different biochemical natures, either secreted or inside extracellular vesicles, and both tumor cells and stromal cells are involved in this process. The mechanisms by which these signals that can modify the premetastatic niche are sent from the primary tumor site include changes in the extracellular matrix, recruitment and activation of different stromal cells and immune or nonimmune cells, metabolic reprogramming, and molecular signaling network rewiring. In this review, we will discuss how hypoxia might alter the premetastatic niche through different signaling molecules.

Keywords: cancer; metastasis; hypoxia; seed and soil; microenvironment; exosomes

1. Introduction

Metastasis is the process by which tumor cells escape from the primary site and establish and reconstitute the tumor in a distant secondary organ. It is a process with multiple stages and is directed by multiple factors, such as organ-, tumor- and immune-related factors. To date, avoiding metastasis formation or eliminating it once it has appeared remains one of the main obstacles to achieve complete remission of cancer after treatment, and metastasis continues to be the leading cause of cancer-related death [1]. In order to
accomplish metastatic colonization, the tumor cells that manage to enter the circulation must find a permissive environment to settle in that stimulates their survival and facilitates their adhesion. This concept, known as the seed and soil theory, was proposed by Stephen Paget as early as 1889 [2]. Therefore, aside from the adaptation of tumor cells to a new environment through tumor plasticity, it is reasonable to think that prior to releasing the tumoral cells into the circulation, the tumor remodels and adapts the secondary site to its needs by sending different factors, such as cytokines, chemokines, enzymes, and growth factors. This view of cancer metastasis as a bidirectional process in which both the disseminating cells and the secondary organ play a key role has been previously described and validated in several cancer types such as breast, colon and melanoma [3]. Recently, it has also been proposed that nasopharyngeal carcinomas may behave as pathological ecosystems, with self-seeding CTCs (Circulating Tumor Cells) releasing soluble factors and exosomes back to the primary tumor site [4]. In recent years, the hypothesis that the hypoxic environment that characterizes tumors may have a direct role in the preparation of the premetastatic niche has been gaining strength, with numerous results supporting it [5,6]. Hypoxia can carry out this function by inducing the release of various molecules, either secreted or inside extracellular vesicles, from both tumor cells themselves and stromal cells [7]. The mechanisms by which these signals that can modify the premetastatic niche are sent by the primary tumor include changes in the extracellular matrix, metabolic reprogramming, and recruitment and activation of different stromal cell types [8]. The present review focuses on the role of the hypoxic-secretome in the preparation of niches for metastasis growth in distant organs. Therefore, we establish a workflow for this review as: (1) hypoxic secretome, (2) hypoxia-related exosomes and (3) effect of hypoxia on cellular components of the premetastatic niche.

2. Hypoxic Secretome

The term “hypoxic secretome” refers to the set of molecules secreted into the extracellular space by a cell, tissue, organ, or organism in response to an environment with low O₂ pressure [9]. Many of these molecules are secreted in exosomes or extracellular vesicles, but others may be liberated as free secreted factors, proteins, cytokines or RNAs (Figure 1).

![Figure 1. Schematic summary. As part of the adaptative response to hypoxic conditions, both tumoral and stromal cells alter the secretion of free molecules and extracellular vesicles, which upon arrival at the secondary site promote a series of changes that result in a permissive premetastatic niche.](image-url)
Among the many molecules released into the environment by the primary tumor under these conditions, there are molecules related to growth and survival (HSP90a (Heat Shock Protein 90a), lncRNA-UCA1, osteopontin and periostin), formation of blood vessels (VEGF (Vascular Endothelial Growth Factor), SDF-1, TGF-β (Tumor Growth Factor β), ANG-1, FGF (Fibroblast Growth Factor), PDGF, and MMPs (Matrix Metalloproteinases)), immune evasion at both primary and secondary sites (CCL5, CXCL12 (Chemokine (C-X-C motif) Ligand 12), IL-10 (Interleukin 10), SDF-1, TGFβ1, VEGF, CCL2), migration (LOX (Lysyl Oxidase), MMPs), intravasation and extravasation (ANGPTL4, CCL2, IL-6, MMPs, PGF, VEGF) and metastatic colonization (LOX, tenasin C, PTHrP (Parathyroid Hormone-related Protein)) [10]. Additionally, many have several effects on the adaptation of the secondary site to make it a hospitable terrain for cells arriving from the primary site. In this review, we briefly describe the effect of the hypoxic secretome on the development of metastasis, with special emphasis on the role that some of these molecules play in establishing a favorable premetastatic microenvironment. See Figure 1 and Table 1.

**Table 1.** Summary of the role of several hypoxia-induced molecules in the generation of the premetastatic niche.

| Molecule | ECM (Extracellular Matrix) Changes | Effect on Premetastatic Niche Preparation | Vascularization |
|----------|-----------------------------------|-----------------------------------------|----------------|---|
| LOX      | Collagen crosslinking ↑Matrix stiffness ↑Fibronectin ↑Osteolysis | CD11b+ BMDCs and MDSCs recruitment ↑Proliferative and invasive phenotype | ↑Angiogenesis |
| VEGF     | ↑Fibronectin ↑Osteolysis and bone reabsorption ↑Bone sialoprotein production MMP-9 secretion | CD11b+ BMDCs and VEGFR+ BMDCs recruitment CXCR2+ tumor cells recruitment Nesting induction ↑Adhesion ↑Invadopodia formation MDSCs recruitment | ↑Angiogenesis ↑Permeabilization |
| TGFβ     | Fibronectin, collagen and periostin production ↑Bone lesions | Immune suppression of NKs, d T cells and CD8+ T cells / N2 and M2 polarization CCL9 expression in CD11b+ BMDCs | / |
| CAIX     | ↑Intregin expression ↑MMP-14 expression ↑MMP-9 secretion | IFNγ production suppression G-CSF production Inhibits NK cytotoxicity CD11b+ BMDCs recruitment | / |
| S100A8 and S100A9 | MMP-9 secretion Versican deposit | Invasive phenotype Increased invadopodia formation Chronic inflammation Mac1+ recruitment | / |
| SAA3     | Osteolysis and bone reabsorption MMP-9 secretion | CD11b+ osteoclastic differentiation Immunosuppressive niche CCL5 expression in LECs | / |
| Global effect | Creates physical space Releases trapped molecules Increases cell adhesion and nesting Difficult access to eliminate tumor cells | Immune suppressed secondary site Increased tumor cell arrival Nutrient arrival to micrometastasis | Favors circulating CSCs arrival |

### 2.1. Lysyl Oxidase

The lysyl oxidase (LOX) family of proteins is made up of a total of five enzymes (LOX and lysyl oxidase-like 1-4) whose expression can be induced by HIF in response to hypoxic conditions. Under physiological conditions, the function of these proteins is to carry out the oxidative deamination of lysine residues in different proteins [11].
According to a study carried out by Erler et al. in 2006, LOX appears to be essential in hypoxia-induced metastasis. Different microarray studies have shown an association between tumor hypoxia levels and LOX expression in tumors such as breast cancer and head and neck cancer. Furthermore, patients with elevated LOX levels show both poor overall survival and poor metastasis-free survival [12,13]. Increased hypoxia-induced LOX secretion is correlated with increased bone metastasis in ER-breast cancer [14]. In epithelial ovarian carcinoma, it is negatively correlated with progression-free survival [15], tumor grade, tumor diameter, and lymph node metastasis [16]. In gastric cancer, LOX levels are correlated with the number of lymph node metastases, greater infiltration depth, and advanced tumor-node-metastasis stages [17]. Elevated LOX expression in hepatocellular carcinoma is correlated with a higher relapse rate and worse survival rate [18]. In lung adenocarcinoma, LOX is part of the hypoxia-related risk signature [19], a correlation that is also observed in non-small cell lung carcinoma [20]. On the other hand, in breast cancer, the most frequent metastatic sites are the bone and the lung, and LOX has been proposed to play an essential role in the development of the premetastatic niche in those specific organs [21].

Therefore, it seems clear that there is a close relationship between LOX expression and secretion under hypoxia and the development of metastases. Recently, several studies have revealed some of the mechanisms through which LOX might exert this function. Some researchers hypothesize that it is due to its participation in migration and invasion via the LOX-FAK-AKT (Protein Kinase B) axis and in EMT (Epithelial to Mesenchymal Transition) via the E-cadherin pathway [22], while others suggest that it is through the establishment of a favorable premetastatic niche. The mechanism would be as follows: under hypoxia, highly metastatic cell lines such as MDA-MB-231 increase the release of nucleotides, specifically ATP, into the tumor interstitium. This ATP is then detected via P2Y2R, a G-protein coupled receptor, resulting in a higher transcription of HIF1α. Once HIF1α expression has been increased, it results in a further increased expression of LOX [23]. In recent years, it has been suggested that LOX may also be regulated by HIF2α, even more strongly than by HIF1α [24]. HIF then stimulates the expression of LOX, which is secreted and transported to a secondary site via the bloodstream. Once there, the enzyme proceeds to induce oxidative deamination of the lysine residues in collagen, allowing collagen crosslinking and the consequent formation of collagen IV fibers [25]. This increases the rigidity of the extracellular matrix and promotes the adhesion of cells that reach the secondary site [11,26]. In High Grade Serous Ovarian Cancer, there is a relationship between extracellular matrix remodeling induced by hypoxia-mediated LOX secretion and peritoneal metastasis [27]. Collagen IV crosslinking favors the recruitment of CD11b+ bone marrow-derived cells (BMDCs) [25,26,28]. Once recruited, BMDCs produce MMP2, which breaks down collagen. On the one hand, this rupture favors remodeling of the premetastatic niche, and on the other hand, it stimulates the arrival of even more BMDCs and tumor cells to the site. BMDCs also secrete angiogenic factors that promote vascularization of the BMDCs and facilitate the arrival of circulating tumor cells [25]. Moreover, when the matrix is stiffer due to collagen crosslinking, there is increased phosphorylation of the FAK/SRC (Steroid receptor coactivator) pathway, leading to the development of a more proliferative and invasive phenotype [29]. Once activated, the FAK/SRC pathway promotes the production of fibronectin. Fibronectin production recruits myeloid-derived suppressor cells, which contribute to the formation of an immune-suppressed premetastatic niche [30]. Finally, LOX promotes osteolytic activity in the bone, an essential event in the formation of premetastatic lesions in this organ [31]. These effects exerted by LOX that arrives via the bloodstream from the primary tumor come as no surprise, since LOX secreted by CAFs (Cancer Associated Fibroblasts) in the hepatic premetastatic niche in gastric cancer contributes to the formation of the niche and is correlated with a worse prognosis [32].
Physiological inhibition of LOX levels by miRNA reduces metastasis and improves prognosis. miRNA29a interacts with the 3’ UTR (Untranslated Region) of LOX, LOXL2 and VEGFA, reducing protein levels. A lower level of miRNA29a is associated with a worse prognosis [33]. Blocking LOX activity with synthetic inhibitors shows a similar effect. Inhibition of the HIF–LOX axis reduces metastasis in orthotopic models of breast cancer [20]. LOX inhibition also appears to block metastasis in pancreatic cancer [34]. The use of LOX inhibitors, such as Pdcd4 and β-aminopropionitrile, decreases the invasive capacity of the breast tumor cell lines T47D and MCF7 [35] and reverses LOX-induced EMT, invasion and metastasis in cervical cancer [36]. However, all these studies focused on demonstrating that LOX inhibition reduces metastatic capacity by preventing it from promoting epithelial–mesenchymal transition. Therefore, in the future, it is necessary to delve deeper into the effect that LOX inhibition has on the development of the premetastatic niche, since there is ample evidence of the role of LOX in this stage of metastasis.

Other proteins in this family, such as LOXL2, also seem to favor metastasis, although to date, studies have focused on pathways other than the formation of the premetastatic niche. For example, LOXL2 seems to promote lung metastasis in breast cancer, possibly by increasing epithelial–mesenchymal transition by stimulating the expression of Snail1 [37]. LOXL2, secreted in vesicles in response to hypoxia, not only acts in the premetastatic niche but can also reach cells of the primary tumor that are not under hypoxic conditions, which helps them carry out epithelial–mesenchymal transition and initiate invasion from nearby tissues. Moreover, the amount of LOXL2 present in extracellular vesicles is higher under hypoxic conditions than under normoxic conditions in head and neck cancer [30]. The secretion of LOXL2 and LOXL4 by breast cancer tumor cells results in increased crosslinking of collagen in the lung, one of the main sites of metastasis for this tumor type [28].

2.2. VEGF

VEGF is a growth factor that induces endothelial cell growth, angiogenesis, and vasculogenesis. VEGF can be induced by hypoxia, as it presents an HIF binding element in its promoter. The activity of VEGF in the neovascularization and permeabilization of blood vessels are its best known roles in metastasis, although it also participates in the establishment of the premetastatic niche [26].

Increased levels of VEGF expression in prostate cancer are associated with the clinical stage, Gleason score, tumor stage, progression, metastasis, and survival. Elevated plasma VEGF levels are correlated with bone metastasis and poor prognosis in many tumor types [38]. In addition, clinical data indicate that VEGFA and tumor-derived VEGFD induce prometastatic lymphangiogenesis and are associated with increased lymph node metastasis [39].

VEGF can exert its effect in preparing the premetastatic niche through different pathways. First, VEGF is capable of activating osteolysis in bone through the BMP (Bone Morphogenetic Protein) pathway. In addition, VEGF is capable of carrying out both autocrine and paracrine signaling in osteoblasts, resulting in the stimulation of bone resorption [38,40–43]. Osteoclasts are the main cells responsible for tumor-induced bone destruction. The resorption of the bone matrix has two different metastasis-promoting effects: it generates a physical space that can be occupied by tumor and stromal cells, and allows the release of growth factors that are trapped within it and that help generate a suitable environment for tumor growth [44,45]. In prostate cancer, activation of osteoblastogenesis is mediated by the VEGF/VEGFR (Vascular Endothelial Growth Factor Receptor) axis through BMP [41]. Another of its actions occurs through stimulation of the production of the proinflammatory cytokines S100A8 and S100A9 at secondary sites, such as the lung, by myeloid and endothelial cells that are already located there before metastasis [46]. In turn, these cytokines remodel the niche through different effects, such as the recruitment of CD11b+ myeloid cells [47]. VEGF also recruits VEGFR1+ BMDCs to fibronectin-rich sites (such as lung and bone). These BMDCs are an essential component in the formation of the premetastatic niche [43] and can stimulate the proliferation and metastasis of esophageal cancer cells in the niche [48]. Once there, BMDCs contribute to extracellular matrix
remodeling through the secretion of MMP-9 [43,49]. There are eight pathways through which recruited BMDCs are estimated to contribute to lung bone metastasis: the ‘T-cell receptor signaling pathway’, ‘osteoclast differentiation’, the ‘MAPK signaling pathway’, the ‘VEGF signaling pathway’, ‘leukocyte transendothelial migration’, ‘signaling pathways regulating the pluripotency of stem cells’, the ‘oxytocin signaling pathway’ and ‘cell adhesion molecules’ (CAMs) [50]. On the other hand, VEGF seems to contribute to bone recognition by tumor cells and to the induction of their nesting [51]. It does so by mediating the expression of adhesion molecules, such as fibronectin and bone sialoprotein, in the extracellular matrix, which can be used by tumor cells to adhere to bone [52]. VEGF also results in increased CXCL5 expression in the lungs (alveolar epithelial cells), which is recognized by CXCR2 (C-X-C Chemokine Receptor) expressed by esophageal cancer tumor cells [48]. Furthermore, VEGF secreted by tumors in mouse mammary glands alters the premetastatic niche and triggers an inflammatory response, inducing the expression of prostaglandin E2 in mouse pulmonary microvascular endothelial cells (MPVECs). This increases the adhesion of breast cancer cells and promotes their organotropism to the lung [53], and increases the activation of invadopodia formation by circulating tumor cells via p38, facilitating adhesion to the premetastatic site [54]. Finally, VEGF plays a key role in the structure of the secondary site vasculature. Neovascularization of the new environment is important not only to facilitate the arrival of circulating tumor cells but also because when circulating tumor cells reach the secondary site, they initially form a micrometastasis that becomes a macrometastasis, requiring neovascularization that allows them to obtain nutrients [55]. VEGF secreted by the primary tumor also increases the permeability of blood vessels in the premetastatic area by inducing Ser490 phosphorylation of occludin, mediating its degradation via the ubiquitin—proteasome pathway and therefore weakening the tight junctions in the blood vessels of the premetastatic niche in the lung in breast cancer [56].

2.3. TGF-β

TGF-β is part of a family of proteins that includes the three protein isoforms TGFβ-1, TGFβ-2 and TGFβ-3 and other functionally related proteins. These proteins play a key role in immune cells and endothelial and epithelial tissues by participating in processes such as survival, proliferation, differentiation, and migration [57]. TGF-β is regulated through hypoxia [58]. Hypoxia induces tumor cell secretion of TGF-β, and contributes to angiogenesis in several tumor types [59].

Blood TGF-β levels appear to be correlated with bone metastasis in prostate cancer patients [60]. Different mechanisms that lead to an increase in TGFβ pathway signaling have been identified as promoters of the formation of the premetastatic niche [57]. One of the more clearly characterized pathways seems to be that of the communication of ovarian cancer tumor cells with the premetastatic niche in the peritoneum. The delivery of TGF-β from the primary to the secondary site induces mesothelial-to-mesenchymal transition in the mesothelial cells of the peritoneum, which seems essential for the development of an adequate premetastatic niche [61]. Similarly, activation of the TGF-β signaling pathway in peritoneal mesothelial cells allows metastasis of gastric cancer to the peritoneum [62]. Osteosarcoma tumor cells send TGFβ packaged in EVs (Extracellular Vesicles) to lung fibroblasts, inducing their differentiation into cancer-associated fibroblasts, which contribute to generation of the premetastatic niche [63]. Activation of the TGFβ-SMAD2/3 pathway in Kupffer cells contributes to gastric cancer metastasis to the liver by causing Kupffer cells to promote activation of the CSC properties of incoming gastric cancer cells [64]. On the other hand, TGF-β induces the expression of CCL9 (Chemokine (C-C motif) Ligand 9) by CD11b+ myeloid cells. CCL9 fundamentally acts as a cell survival factor that allows the survival of tumor cells during the metastatic process. Furthermore, signaling of CCL9 through CCR1+ (C-C Motif Chemokine Receptor 1) results in the recruitment of myeloid progenitor cells that facilitate tumor cell invasion [65].
In addition, TGF-β promotes an immunosuppressive niche by inhibiting NK (Natural Killer) cells, γδ T lymphocytes, and CD8+ lymphocytes while promoting the polarization of neutrophils and macrophages to N2 and M2, respectively. TGF-β also stimulates CCR1+ myeloid cells, which secrete MMPs that remodel the premetastatic niche [66]. The effect of TGFβ on immune evasion during liver metastasis has been recently demonstrated by Taurellio et al. In their murine model, liver metastases exhibited high levels of TGFβ in the stroma along with a low rate of T-lymphocyte infiltration [67]. Blocking TGFβ signaling with inhibitors increased T-cell activity in clearing tumor cells and resulted in a reduction in the appearance of metastases in the early stages [67].

On the other hand, TGFβ secreted by Kupffer cells activates HSCs, causing the production and deposition of collagen-1 and fibronectin. This fibrous environment produces a greater recruitment of bone marrow-derived macrophages and granulocytes while acting as a physical barrier that makes it difficult for the immune system to eliminate tumor cells [57]. In salivary adenoid cystic carcinoma metastases, vesicles secreted by the primary tumor containing α2β1 integrins are taken up by lung fibroblasts, causing them to secrete TGFβ. TGFβ increases the remodeling capacity of the microenvironment at the secondary site, in part through the production of peristin. Peristin is a protein that binds collagen and fibronectin and activates the AKT and STAT3 signaling pathways, thereby recruiting myeloid suppressor cells and creating an immunosuppressive environment in the secondary organ [69]. Finally, TGFβ1 produced by platelets in prostate cancer seems to affect the osteoclastogenesis/osteoblastogenesis balance, resulting in bone lesions that favor the development of a premetastatic microenvironment [70]. Although many of these studies do not directly describe the role of TGFβ secreted by the primary tumor, they do seem to suggest that if TGFβ from other sources can have such a high impact on premetastatic niche formation, it could be expected that TGFβ secreted in response to hypoxia could act similarly, making it an interesting avenue for future research.

2.4. CAIX

The carbonic anhydrase (CA) family of metalloenzymes includes numerous isoforms and is responsible for catalyzing the conversion of carbon dioxide to bicarbonate anion [71]. Under hypoxic conditions, HIF-1 translocates to the nucleus and activates the expression of carbonic anhydrase IX (CAIX) [72]. In cancer, the increase in CAIX levels in response to hypoxia is a mechanism of the tumor response to stress caused by low oxygen pressure. CAIX functions as a mediator of tumor growth and metastasis [73].

Multiple studies have shown a correlation between CAIX and poor prognosis or increased metastasis [74,75]. In thyroid cancer, patients with higher CAIX expression have a higher frequency of lymph node metastases [76]. The use of numerous combinations of CAIX inhibitors reduces the metastatic capacity of tumor cells in various models [77]. For example, CAIX promotes cell motility in cervical cancer through the ERK/PFKFB4 pathway [78], its inhibition in triple-negative breast cancer reduces lung metastasis [75], and suppression of the HIF1α/CAIX axis in epithelial ovary cancer reduces malignancy and invasion [79].

CA contributes to virtually all stages of tumor development and progression [80]. Regarding the ways in which CAIX participates in the development of metastases, CAIX levels are correlated with the expression of various integrins and MMP14, which are necessary for pseudopodia/invadopodia formation and degradation of the extracellular matrix at the beginning of the metastatic process [81]. CAIX overexpression increases MMP9 expression and FAK and steroid receptor coactivator (Src) phosphorylation, promoting tumor cell motility in oral squamous cell carcinoma [82]. However, currently, only one mechanism has been described through which CAIX seems to influence the establishment of the premetastatic niche. HIF1α promotes G-CSF production in breast cancer cells by activating the carbonic anhydrase axis CAIX-NFkB-G-CSF. G-CSF mobilizes granulocytic CD11b+Ly6G+Ly6C+ myeloid-derived suppressor cells (MDSCs) to the lung [83], where they produce Bv8 protein to induce premetastatic niche formation [84]. CD11b+Ly6G+Ly6C+ cells can also suppress...
IFNγ (Interferon γ) production and NK cell cytotoxicity in the niche [85]. Targeting G-CSF appears to reduce metastasis by partially preventing premetastatic niche formation [83]. G-CSF can be shipped within exosomes and is involved in increased angiogenesis and vascular permeability [86]. In addition, it is believed that G-CSF may play a role in the generation of metastatic lesions by cancer stem cells [87,88].

2.5. S100A8 and S100A9

S100A8 and S100A9 are two proinflammatory cytokines through which many of the molecules secreted by the primary tumor in response to hypoxia exert their function. For example, TNFα (Tumor Necrosis Factor α) and TGF-β, together with VEGFA, induce the expression of S100A8 and S100A9 in the lung, promoting development of the premetastatic niche [46].

Moreover, S100A8 and S100A9 induce the transcription of serum amyloid A (SAA) 3, which acts as a chemoattractant for Mac1+ myeloid cells in premetastatic sites through the activation of NKκB via TLR4 (Toll Like Receptor 4). However, the specific molecular mechanism remains unknown. The accumulation of Mac1+ at the premetastatic site generates an inflammatory-like state which accelerates the arrival of tumor cells. Furthermore, SAA3 establishes a positive feedback loop by which it enhances its own secretion via TLR4 [46]. SAA3 also maintains a state of chronic inflammation at the secondary site [89] and plays an important role in metastatic niche formation [90]. SAA3 protein produced in response to S100A8 in the lungs also attracts and recruits circulating tumor cells to the niche [46] and stimulates the production of inflammatory cytokines, such as TNFα [89], which promote tumor growth [91]. S100A8 and S100A9 also recruit CD11b+ myeloid cells to the niche [54], where they deposit versican in the extracellular matrix [92] and secrete large amounts of MMP9 [93].

2.6. IL-6

Although IL-6 is often not considered in the effect of the hypoxic secretome on the formation of the premetastatic niche, there are multiple lines of evidence indicating that IL-6 could be a molecule of interest in future research in this area.

A recent study showed that the IL6/STAT3 pathway orchestrates the formation of an immunosuppressive premetastatic niche in the lungs. IL-6 deregulation in tumor cells reprograms the STAT3 pathway in metastatic cells and promotes the recruitment of myeloid suppressor cells and macrophage polarization toward a phenotype that allows tumor cells to escape immune system surveillance [94]. Furthermore, it was previously shown that breast cancer tumor cells secrete IL-6, which causes STAT3 Y705 phosphorylation in the lymphatic endothelial cells of lymphatic vessels. This phosphorylation activates HIF1α and VEGF and the expression of CCL5 in LECs (Lymphatic Endothelial Cells), directing the spread of the tumor to other tissues [95]. On the other hand, IL-6 is one of the main cytokines involved in the formation of the bone premetastatic niche through bone remodeling. Binding of IL-6 to its receptor activates the JAK/STAT pathway and promotes osteoblast differentiation towards a mature phenotype [96]. IL-6 secreted by both tumor cells and stromal cells is involved in the induction of osteoblastogenesis and bone resorption, which are key processes in the formation of the premetastatic niche in bone [97–100]. PC3 conditioned medium, rich in IL-6 and IL-8, induces the osteoclastic differentiation of CD11b+ blood cells and bone resorption [101]. Suppression of IL-6 expression in combination with clodronate liposome treatment in PC3 cells reduces osteoclast formation and reduces lymph node metastasis in a mouse model of metastasis [102]. Furthermore, the induction of IL-6 by macrophages under hypoxic conditions enhances the metastatic and invasive capacity of cancer cells [103]. Finally, the secretion of IL-6 by endothelial cells in the tumor microenvironment is also capable of activating MMP9 and causing tumor remodeling [104].
2.7. Other Molecules

The roles of other molecules secreted by the primary tumor in response to hypoxia in the formation of the premetastatic niche are not well known but would be interesting to consider (Table 1).

PTHrP secretion by breast cancer cells can be induced by HIF2α [105], and this secretion is related to an increase in osteolytic lesions and bone metastases and to increased bone marrow colonization [106].

CXCL12 can be induced by hypoxia and is known to promote breast cancer dissemination via CXCR4 signaling [107]. Among the factors secreted by primary tumor cells in response to hypoxia, CXCL12 could be an interesting factor because other functions are known to be exerted in the premetastatic niche when CXCL12 is secreted locally. For example, when CXCL12 is secreted by endothelial cells, it attracts CXCR4-expressing cells into the CXCL12 gradient, facilitating intravasation [108]. On the other hand, the induction of CXCL12 expression in the premetastatic niche by factors secreted from the primary tumor is also involved in the homing of tumor cells to the secondary site. As an example, in prostate cancer, the transfer of pyruvate kinase 2 from the primary tumor to bone marrow stromal cells results in the increased expression of CXCL12 in the bone marrow in an HIF1α-dependent manner, favoring the establishment of metastatic cells in bone [109].

Matrix metalloproteinases constitute another interesting group of molecules. MMP9 is produced in response to VEGF and PIGF (Placenta Growth Factor) released by the primary tumor, and is responsible for breaking down the extracellular matrix to weaken the physical barrier that it constitutes, simultaneously releasing growth factors and soluble molecules that are trapped within it [49]. In addition, MMP13 is induced by HIF1α and is capable of breaking down pro-MMP9 to produce active MMP9 [5]. On the other hand, several of the molecules that arrive at the secondary site result in the recruitment of CD11b+ BMDCs. MMP9 secreted by VEGFR+ and VLA4+ (Very Late Antigen 4) BMDCs contributes to extracellular matrix remodeling during premetastatic niche formation [110]. Mac1+ Cd11b+ cells recruited to the niche by LOX also produce MMP2, which lyses collagen and releases collagen IV chemokattractant, thereby recruiting more CD11b+ cKit+ BMDCs and metastatic cells [7].

Other molecules, such as osteopontin, periostin, tenascin and CKB (Creatine Kinase Brain-type), may also play an important role in the development of the premetastatic niche, despite the little information that is available to date [10]. Periostin in metastatic niches serves to concentrate and present Wnt ligands, thereby inducing and maintaining the stem-like properties of founder cells that reach a metastasis site [7]. Tenascin C is secreted by hypoxic breast tumor cells and promotes lung colonization by altering the Notch and WNT signaling pathways [111]. Tumor cells secrete brain-type creatine kinase (CKB) in response to hypoxia as a mechanism of adaptation to metabolic stress under these conditions. CKB generates phosphocreatine, which can subsequently be used by tumor cells to obtain ATP [112].

In the upcoming years, new secreted molecules could be taken into consideration for their effect on premetastatic niche formation. GRP78, or 78-kDA glucose-regulated protein, is a protein belonging to a group of heat shock proteins related to the response to the unfolded protein response. Its expression is significantly increased through HIF1α (Hypoxia-Inducible Factor α) under hypoxic conditions [113]. This molecule has already been shown to promote an immunosuppressive tumor microenvironment after being secreted by tumor cells by regulating the production of cytokines by dendritic cells and macrophages [114]. Recently, a study by Chen et al. in 2020 showed that GRP78 participates in the development of immunotolerance in the premetastatic niche. This effect is mediated by the modulation of the activity of macrophages and dendritic cells, the suppression of liver-resident NK cells, and the induction of TGF-β expression [115]. Additionally, in 2020, miRNA92a (regulated by hypoxia through HIF1α) [116] was reported to be released by BMDCs recruited to the premetastatic niche in response to hypoxia. Once secreted, miRNA92a acts by inhibiting its target SMAD7, leading to increased TGFβ secretion by hepatic stellate cells. Elevated levels of this miRNA have been found in the serum of lung
cancer patients. Therefore, it is possible that miRNA92a also plays a role in premetastatic niche adaptation, although more research is needed [117].

3. Exosomes

Communication between cells in the tumor microenvironment and the premetastatic niche can occur not only through the secretion of soluble molecules but also through the release of molecules inside extracellular vesicles, such as exosomes [118]. Exosomes are extracellular vesicles generated by the invagination and budding of the endosomal membrane and are later released during the fusion of multivesicular endosomes [119].

Communication via tumor-derived vesicles (TDVs) is well known to play a key role in metastasis development in several tumor types, including nasopharyngeal [120,121], oral [122], bladder [123], lung [124], prostate [125], breast [126], pancreatic [127], and ovarian [128] cancer. Furthermore, the secretion of tumor-derived exosomes into the bloodstream has previously been considered a promoter of the formation of the premetastatic niche [129]. For example, exosomes derived from melanoma cells reprogram progenitor cells from the bone marrow and promote vascular leakiness at the premetastatic niche [130].

In recent years, hypoxia has been demonstrated to affect both the number of extracellular vesicles produced by tumoral and stromal cells and the molecular composition of their cargos [131].

The content of TDEs (Tumor Derived Exosomes) can extensively change the landscape of the premetastatic niche. HIF1α is one of the most relevant cargos found in these vesicles and can be sent from one cell to another inside vesicles without its DNA-binding and transcriptional activity being compromised. Delivery of HIF1α results in an alteration of EMT-associated proteins in the receptor cell, such as E- and N-cadherin [120]. Other important proteins for premetastatic niche progression that can be delivered via TDVs are matrix metalloproteinases: the presence of MMP-13, MMP14 and C4.4A within TDVs increased under hypoxia. Moreover, exosomal MMP-13 can increase the expression levels of vimentin while reducing E-cadherin expression in recipient cells [121]. In addition to matrix remodeling enzymes, several soluble factors have also been found inside TDEs, including TGF-β, TNF-α, IL-6, and IL-10, all of which play a key role in regulating cell migration [124,125].

Proteins do not constitute the sole cargo of TDEs: a plethora of noncoding RNAs can also be packed in these vesicles. HIF1α activation results in an enrichment of several miRNAs as exosome cargos, such as miR-21-3p, miR125b-5p and miR181d-5p in ovarian cancer [132] and miR-135b and miR-21 in melanoma [133] and PANC cells [134]. Once it reaches its target cell, miR-21 triggers prometastatic phenotype polarization [122]. The delivery of lncRNA-UCA1 by TDEs from bladder cancer cells also affects EMT [123]. In general, the levels of miRNA loaded into exosomes are significantly higher under hypoxic conditions than under normoxic conditions [122].

In the literature, hypoxia has been reported to induce the production of exosomes in different cancer cell lines, including breast cancer cell lines and oral squamous cell carcinoma cell lines [122,135]. Furthermore, several studies support the hypothesis that hypoxia increases the production of exosomes by tumoral cells compared to normoxic conditions [131]. Among the various ways in which HIF1α affects the production and release of exosomes, we found an upregulation of Rab7 and Rab27a via STAT3 [136], an increase in the expression of Rab22a and small GTPase Rab22a [126], an accumulation of Rab5 [137] and a rearrangement of cytoskeleton fibers through ROCK [138]. In addition, the presence of a hypoxic environment results in an increase in calpain expression, which has been suggested to cause the shedding of microvesicles [139]. Another effect of hypoxia is a reduction in the production of ceramide, which in turn promotes the biogenesis of ILVs, from which exosomes are then derived [140]. See Figure 2.
3.1. Effect of Hypoxic Exosomes on Stromal and Immune Cells

Tumor cells are not the only cells affected by the receipt of TDEs. The activity of stromal cells can also be affected by the receipt of several cargos [141].

TDEs have several effects on the biology of endothelial cells. They have been shown to play a role in the potentiation of angiogenesis [142] in cancer types such as glioblastoma (GBM), where hypoxic microvesicles containing tissue factor and factor VIIa promote endothelial cells to acquire an angiogenic phenotype [143] while stimulating microvessel sprouting. Furthermore, the release of carbonic anhydrase within TDEs from hypoxic renal cell carcinoma cells promotes endothelial cell migration and tube formation [86]. On the other hand, miR-210 secreted from leukemia cell lines induces tubulogenesis [144]; miR-135b inhibits FIH-1 (Factor Inhibiting HIF-1) and stimulates endothelial tube formation [133]; miR-494 from lung cancer cells targets PTEN, activating the AKT/eNOS pathway and resulting in enhanced angiogenesis; and miR-23a promotes angiogenesis by targeting prolyl hydroxylase and thus avoiding the degradation of HIF-1α [145].

In the case of fibroblasts, TDEs can promote polarization toward a cancer-associated phenotype (CAFs) in different ways, including the induction of α-SMA expression [125]. MicroRNA and proteins delivered by TDEs are internalized and result in the activation of several pathways that ultimately lead to a CAF-like phenotype [146]. However, a lot of specific information is missing on how this happens; thus the importance of highlighting the potential relevance of these processes.

TDEs also show the ability to generate an immunosuppressive environment and facilitate immune escape.

For example, TDEs can regulate T-cell activity by inducing CD8+ T cell apoptosis [147]. TDEs from S-180 cells and Lewis lung carcinoma cells downregulate CD4+ T cells and promote Treg expansion via miR-214 [148]. Treg expansion can also be achieved through the delivery of miR-208 inside exosomes [149]. Likewise, TDEs can induce Treg expansion and impair Th1 and Th17 differentiation by targeting fibroblast growth factor 11 due to miR-24-3p released within the vesicles [150]. Tumor exosomes reduce T cell cytotoxicity and proliferation through a mechanism that involves HSP70 [151]. The transfer of miR-21 within these vesicles amplifies the inhibitory effect that MDSCs have on T cells through...
the PTEN/PD-L1 pathway [151]. In addition, T cells that receive TGF-β from TDEs under hypoxic conditions lose their function [152].

The arrival of exosomes from cancer cells to macrophages induces polarization toward the M2 phenotype through the release of miR-940, miR-21-3p, miR125b, and miR-181d-5p and the blockade of PTEN/PI3Kgamma via miR-301a-3p [153]. The same is true regarding the delivery of miR-103a and polarization toward the M2 phenotype through activation of the AKT/STAT3 pathway [154].

The activity of NK cells is impaired in the hypoxic tumor microenvironment via several mechanisms [155]. Exosomal TGF-β secreted from lung cancer cells under hypoxia regulates the antitumoral response by decreasing the expression levels of NKG2D in NK cells, thereby reducing the activation of this cell type [156]. This exosomal TGFβ can also impair the cytotoxic activity of NK cells [157]. Moreover, miR-23a targets CD107a and consequently blocks NK cell activity [157], and circUHRF1 carried within exosomes is able to inhibit miR-449c-5p and, as a consequence, upregulate TIM3, which plays an immunosuppressive role [158].

In myeloid-derived suppressor cells, TDEs are able to promote MDSC immunosuppressive activity via heat shock protein Hsp72 [159] or Toll-like receptor 2 [160], and they also induce MDSC expansion and activation through miR-10a and miR-21 [161].

### 3.2. Effect of Hypoxic Exosomes from Stromal Cells on Tumor Cells

Exosomes derived from stromal cells have an impact on the behavior of tumor cells, making this cellular communication bidirectional. Hypoxic exosomes derived from macrophages deliver miR-223, which targets the Mef2c/β-catenin pathway and, as a result, promotes cancer invasiveness [162]. The delivery of apolipoprotein E by these vesicles is also able to promote cancer cell migration [163]. CAF-derived exosomes contain miR-21, which binds to APAF1 (Apoptosis Protease-Activating Factor-1) inside tumor cells and, as a result, suppresses apoptosis [164]. However, evidence that hypoxia effectively modifies CAF-derived exosome function is still lacking. The same is true for MSC-derived exosomes, for which very little evidence is currently available.

### 3.3. Effect of Hypoxic Exosomes from Stromal Cells on Stromal Cells

The effect of stromal-derived vesicles is also noticed in other stromal cells. As an example, the impact of DC-derived exosomes on other stromal cells is still being studied, and at the moment there is not enough evidence. Exosomes released by macrophages that contain miR-223 induce the differentiation of other monocytes toward macrophages [165]. Once again, there are not enough studies on MDSC-derived exosomes under hypoxia and their effect on other stromal cells.

### 4. Effect of Hypoxia on Cellular Components of the Premetastatic Niche

#### 4.1. Effect of Hypoxia on Cellular Senescence and SASP Induction

Senescence is a cellular state characterized by an increase in p16 INK4A levels that results in cell cycle arrest [166]. It can be triggered by different stressors [167], including hypoxia [168].

Cellular senescence results in the increased expression and release of multiple secreted factors, which collectively are known as the senescence-associated secretory phenotype [169]. The factors secreted by senescent cells vary depending on the tissue and cell type, although at least 55 genes have been identified as common among all senescent cells [170], including VEGF, PDGF, HGF, IL1a, IL6, IL8, IL10, IL13, IL15, MMP3, MMP9, CXCL1, CXCL2, CXCL5, CXCL11, CXCL12, CCL2, and CCL20 [166].

The SASP (Senescence Associated Secretory Phenotype) can be divided into insoluble components, secreted proteases, and soluble signaling factors (interleukins, chemokines, and growth factors). Soluble factors include IL-6 [171], IL-1 [172], CCL2, G-CSF (Granulocyte Colony Stimulating Factor) [171] and GM-CSF (Granulocyte and Monocyte Colony
Stimulating Factor). Among the secreted proteases, we can find several MMPs [169]. Insoluble components are released inside exosome-like small extracellular vesicles [173–177].

The expression of many SASP factors is regulated by activation of the NF-Kb and C/EBPbeta pathways during senescence [178,179]. Some of them also have positive feedback loops that regulate their expression, such as IL-1α, which regulates its own synthesis and is also a positive regulator of both IL-6 and IL-8 [180]. SASP secretion is also regulated by different miRNAs, such as miR-146a/b, which negatively regulate IL-6 and IL-8 secretion [181].

As we described in the previous paragraphs, many of these molecules have been associated with the preparation of the premetastatic niche. Furthermore, the SASP has several paracrine effects on neighboring cells, including promotion of angiogenesis [171], EMT, invasion [182] and metastasis [183]. Therefore, it is expected that the induction of senescence, and by extension the SASP, results in an enhanced PMN (Pre Metastatic Niche) preparation.

The role of IL-6 released by senescent cells has been particularly well described. First, IL-6 seems to have a key function in the induction of proliferation [184], EMT [185–187] and migration, most likely via STAT3 activation [188]. Breast cancer cell lines have been demonstrated to migrate more easily if cocultured with senescent cells [171]. This ability seems to be mediated by IL-6 and IL-8, both of which promote MMP expression [188,189]. Disruption of the basal membrane by matrix metalloproteases also enhances invasion and metastasis [174]. Additionally, IL-6 disrupts cell adhesion, thereby promoting invasion [190]. IL-6 also promotes tumor angiogenesis [191].

On the other hand, we have serum amyloid A (SAA), which is an acute phase protein that the liver secretes after injury or infection, although in recent years, different studies have reported that it can also be produced by cancer cells and cancer-associated cells. In this case, the production of SAA promotes cancer progression and metastasis [192]. SAA transcription is induced by the JAK/STAT3 pathway upon the interaction of IL-6 with its receptor. Alternatively, the binding of TNFα, IL-18 or IL-1β to their corresponding receptors also results in increased SAA expression via the NFkB pathway. Furthermore, IL1β displaces a constitutive repressor of NFkB signaling, further increasing the overexpression of SAA [193]. Notably, both IL-6 and IL-1b are molecules produced by senescent cells as part of the SASP. In turn, SAA expression results in the production of several proinflammatory cytokines by receptor cells [193]. SAA boosts the invasiveness capacity of tumor cell lines. For example, in renal cell carcinoma cell lines, SAA promotes MMP9 expression, and in glioma cell lines it induces the secretion of not only MMP9 but also of IL-8 and ROS (Reactive Oxygen Species) [194,195]. Several studies have noted that SAA might execute its role in cancer progression through autophagy regulation, potentially through the PI3K/AKT and MAPK pathways [192].

Fibronectin (FN) overexpression is likewise associated with senescence triggered by external stressors, such as hypoxia [196]. Furthermore, the folding of this protein might be compromised due to several factors, which also include hypoxia [197]. FN seems to promote metastasis [198] and is a well-known marker of the mesenchymal phenotype, which points toward the possibility that HIF1α overexpression under hypoxia might result in re-expression of FN in tumor cells, which promotes EMT. However, it is still not clear whether FN expression is a result or a consequence of metastatic initiation [199,200]. Matrix stiffness can seriously affect the metastatic process [201], and the following hypothesis has been proposed: FN allows a softer ECM that facilitates tumor vascularization for tumor growth until hypoxia slowly erases FN and stiffens the ECM upon the crosslinking of collagen induced by LOX. This promotes vascular abnormalities and therefore enhances metastasis [202,203]. This fact might seem contradictory to the previous affirmation that ECM destruction facilitates metastasis. However, both processes are not mutually exclusive, nor dependent on each other. The destruction of the ECM (specifically on the bone) generates a physical space that can be more easily occupied by the arriving cells. Furthermore, the destruction of ECM also releases molecules that were trapped inside and
that may act as chemoattractants or nutrients. Once the cells arrive in the premetastatic niche, if the remaining ECM is stiffer than usual, it will be easier for the circulating tumor cells to attach to it.

FN may also facilitate secondary site colonization: CTCs become more resistant to anoikis by forming clusters in a process mediated by plakoglobin [204–206]. Plakoglobin stabilizes FN mRNA, facilitating perifN assembly on primary tumor cells and therefore cluster formation [207]. Last, but not least, tumor cells from the primary site secrete chemoattractants and exosomes containing stimulatory factors that likely increase FN expression in recruited TAMs (Tumor Associated Macrophages) and CAFs, which results in ECM deposition and remodeling [43,208]. This results in VEGFR1+ BMDC recruitment and adhesion to FN through integrin α4β1 expressed on their surface [43]. Apparently, pro-tumor inflammatory chemokines and cytokines are characteristics shared among the TME, metastatic niche and premetastatic niche, resulting in the accumulation of polymerized FN that promotes tumor cell growth [54].

Finally, many of the SASP components act as regulators of the immune system and, as a consequence, have a remarkable effect on cancer progression. While the SASP may promote clearance of senescent cells and improve immunosurveillance when acutely activated [209–211], the chronic presence of the SASP promotes invasion and metastasis [182] due to the establishment of an immunosuppressive microenvironment [212].

The role of the SASP in premetastatic niche preparation has been described for several cancer types. For example, senescent osteoblasts that secrete IL-6 cause increased bone resorption, which leads to a more suitable environment for breast cancer cells to metastasize [213]. This suitability is most likely achieved through the recruitment of myeloid-derived suppressor cells by IL-6 [214]. In pancreatic cancer, FGF seems to drive cancer dissemination [215,216], and in breast cancer, IL-6 and IL-8 released by senescent fibroblasts increase invasiveness in several cell lines [171]. In addition, MMP2 and MMP3 secretion promotes invasion [169]. IL-1 activates the endothelium, thus increasing the ability of cancer cells to attach to blood vessels [217]. Finally, SASP components might be of particular interest due to their potential role in cancer cell dissemination in ovarian cancer, specifically through the expression of IL-6, IL-8 and MMPs. IL-6 increases the number of ovarian CSCs [218], stimulates EMT [219], and acts as an immunosuppressive cytokine [220]. On the other hand, IL-8 facilitates EMT, and high levels of this cytokine are correlated with greater cancer dissemination [221].

4.2. Effect of Hypoxia on CSCs

CSCs constitute a small subpopulation of cancer cells that present a range of stem cell-like properties, such as self-renewal capacity, quiescence, and a slower cell cycle [222,223]. They are responsible for processes related to poor prognosis, such as therapy resistance, metastasis and tumor relapse [224].

CSCs actively remodel their niche by secreting a range of factors that recruit other cells and modify the extracellular matrix characteristics [225]. In 2020, Lopez de Andrés et al. compiled a summary of the principal CSC secretome components that regulate those processes [224]. Among the molecules that constitute the CSC secretome, we can find several interleukins (IL-6, IL-8, IL-1β), multiple MMPs and VEGF, all of which may be secreted either in a soluble form or inside tumor-derived vesicles [226–228]. Other molecules, such as HIF1 [120], TGF-β [229–231], CCL2 [232] and miRNAs [233,234], are released into the bloodstream encapsulated inside exosomes.

The CSC secretome is able to interact with and modify the premetastatic niche at distal sites [234]. As an example, VEGF enhances the permeability of blood vessels surrounding the PMN [235]. Moreover, VEGF is also able to increase MMP-9 expression in cells in the secondary organ, which helps remodel the ECM [49]. In addition, BMDCs can be recruited by VEGF signaling [43]. Once they arrive, the BMDCs respond to TGFβ signaling by releasing CCL9, which helps generate a protumoral microenvironment [65]. Both VEGF and TGFβ stimulate the production of several inflammatory chemoattractants in the secondary
Cancers 2022, 14, 5930

On the other hand, CSCs are known to show high levels of CXCR4 on their surface, which helps them recognize CXCL12. As a consequence, CXCR4/CXCL12 signaling may guide the migration and colonization of the secondary organ [236–238].

Hypoxia activates the transcription of HIF-1α and HIF-2α in CSCs [239,240], which in turn promotes the transcription of IL-6 and IL-8 [241,242]. IL signaling is a key factor in the upregulation of pathways such as Wnt/β-catenin, NFkB and SMADs, which are all required for the expansion of CSCs [218,243,244]. Furthermore, signaling by TGF-β, which is known to be secreted under hypoxia, has been demonstrated to activate IL-8 production by CSCs, resulting in an enrichment of that cell subpopulation [245]. Moreover, the secretion of several molecules encapsulated in exosomes is enhanced under hypoxic conditions and has also been reported to promote PMN formation [85,246]. Therefore, hypoxia might also contribute to PMN development by enhancing the survival and colonization chances of CSCs that leave the primary tumor and arrive at the secondary site.

CSCs from the primary tumor can be found in the premetastatic niche in both dormant and active states. The transition from one state to the other is determined by the stimuli received by the CSCs in the premetastatic niche [237]. Dormancy has been described as a common feature of cancer cells derived from a hypoxic microenvironment, and IL-6 expression has been reported to be related to a dormant phenotype [247]. The expression of thrombospondin in the perivascular niche maintains CSCs in a dormant state, and the production of tenascin C and fibronectin by endothelial cells accelerates the growth of CSCs [248]. CSCs can induce the production of these molecules by stromal cells, and once they receive their signal, pathways such as the Wnt, Nanog and Oct4 pathways are activated, allowing the CSCs to leave the dormant state [249,250]. Another way in which CSCs can leave the dormant state is through the action of fibronectin and type I collagen, which stimulate the ERK/MAPk pathway and beta1-integrin signaling [251,252]. The production of these molecules (tenascin C, fibronectin, etc.) that allow CSCs to leave the dormant state has been related to hypoxia, suggesting that hypoxia may also promote metastasis development by allowing CSCs at the secondary site to leave the dormant state.

CSCs at the secondary site also interact with stromal cells in the secondary organ to promote metastasis. Several cytokines released by cells within the tumor microenvironment determine the polarization of tissue macrophages, polarizing them towards a cancer-associated phenotype [253,254]. Some of these signaling molecules come from the cancer cells themselves [255]. After being polarized, tumor-associated macrophages (TAMs) secrete IL-6 [256], which binds to its receptor on CSCs and activates the STAT3 pathway, promoting CSC expansion and activation of the expression of stem-related genes, such as Sox-2, Oct-3/4 and Nanog. This has been verified in breast cancer stem cells and lung cancer cells [257]. The arrival of IL-6 also enhances breast CSC migration, promotes tumor growth and metastasis [258], and induces angiogenesis by inducing the expression of proangiogenic molecules, such as VEGF, MMP2 and MMP9 [259,260]. As another example of this type of interaction, breast cancer cells that arrive in the bone secrete osteopontin, which causes the differentiation of bone resident fibroblasts into myofibroblasts, promoting cancer progression [260]. The secretion of osteopontin also polarizes fibroblasts toward the CAF phenotype, and in turn, the CAFs release periostin to support cancer growth in the metastatic niche [237,249].

This evidence suggests that CSCs may have a revealing role in the formation of the PMN, especially when stimulated by a hypoxic environment in the primary organ, although further research is clearly needed if we are to understand the underlying mechanisms of this complex process [224].

5. Conclusions

During tumor growth, the delivery of oxygen to cells is hampered by aberrant or absent vasculature that results in hypoxia. This causes an adaptive response that activates the expression of genes that control several essential processes. Hypoxia activates the transcription of HIF-1α and HIF-2α, which in turn induces CSC expansion by promoting
the transcription of secreted proteins, cytokines, non-coding RNAs and chemokines that will promote the adaptation of niches in different organs to receive, promote survival and adapt to proliferate malignant cells from the primary tumor, especially CSCs capable of regenerating the bulk of the tumor mass in the new emplacement, forming metastasis (Figure 3). CSCs at the secondary site also interact with stromal cells in the secondary organ to promote metastasis. Several cytokines released by cells within the secondary tumor microenvironment determine the tissue inflammatory infiltration and the cancer-associated phenotype of the immune component. Therefore, hypoxia initiates a cascade of physiological responses that not only affect the primary tumor, but also prepare secondary niches in distant organs for the apparition and development of metastasis.

![Figure 3. Effect of hypoxia on cellular components of the premetastatic niche.](image)

**Author Contributions:** Conceptualization, C.C.-G. and A.C.; investigation, C.C.-G. and A.C.; data curation, C.C.-G. and A.C.; writing—original draft preparation, C.C.-G.; writing—review and editing, C.C.-G. and A.C.; supervision A.C.; funding acquisition, A.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by grants from the Ministerio de Ciencia, Innovación y Universidades (MCIU) Plan Estatal de I+D+I 2021, a la Agencia Estatal de Investigación (AEI) y al Fondo Europeo de Desarrollo Regional (MCIU/AEI/FEDER, UE): RTI2018-097455-B-I00, ID2021-12269OB-I00; grant from the AEI-MICIU/FEDER (RED2018-102723-T); from CIBER de Cáncer (CB16/12/00275), co-funded by FEDER from Regional Development European Funds (European Union); from Consejería de Salud (PI-0397-2017) and Project P18-RT-2501 from a 2018 competitive research projects call within the scope of PAIDI 2020, co-financed by the European Regional Development Fund (ERDF) from the Regional Ministry of Economic Transformation, Industry, Knowledge and Universities. We also thank the Fundacion AECC for supporting this work (Funding Ref. GC16173720CARR). CC-G is a recipient of an FPU fellowship from AEI-MICIU. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

**Conflicts of Interest:** The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.
Abbreviations

AKT Protein Kinase B/AKT
APAF1 Apoptosis Protease-Activating Factor-1
BMDCs Bone Marrow-Derived Cells
BMP Bone Morphogenetic Protein
CAFs Cancer Associated Fibroblasts
CAIX Carbonic Anhydrase IX
CAMs Cell Adhesion Molecules
CCL9 Chemokine (C-C motif) Ligand 9
CCR1 C-C Motif Chemokine Receptor 1
CKB Creatine Kinase Brain-type
CSCs Cancer Stem Cells
CTCs Circulating Tumor Cells
CXCL Chemokine (C-X-C motif) Ligand 20
CXCR C-X-C Chemokine Receptor
ECM Extracellular Matrix
EMT Epithelial to Mesenchymal Transition
EV Extracellular Vesicles
FGF Fibroblast Growth Factor
FIH-1 Factor Inhibiting HIF-1
FN Fibronectin
GBM Glioblastoma Multiforme
G-CSF Granulocyte Colony Stimulating Factor
GM-CSF Granulocyte and Monocyte Colony Stimulating Factor
GRP78 78-kDA glucose-regulated protein
HIF Hypoxia-Inducible Factor
HSP Heat Shock Protein
IFN Interferon
IL Interleukine
LECs Lymphatic Endothelial Cells
LOX Lysyl Oxidase
MDSCs Myeloid-Derived Suppressor Cells
MMP Matrix Metalloproteinase
MPVECs Microvascular Endothelial Cells
NK Natural Killer
PIGF Placenta Growth Factor
PMN Pre Metastatic Niche
PTHrP Parathyroid Hormone-related Protein
ROS Reactive Oxygen Species
SAA Serum Amyloid A Protein
SASP Senescence Associated Secretory Phenotype
Src Steroid receptor coactivator
TAMs Tumor Associated Macrophages
TDEs Tumor Derived Exosomes
TDVs Tumor Derived Vesicles
TGF-β Tumor Growth Factor beta
TLR Toll Like Receptor
TNFα Tumor Necrosis Factor alpha
UTR Untranslated Region
VEGF Vascular Endothelial Growth Factor
VEGFR Vascular Endothelial Growth Factor Receptor
VLA4 Very Late Antigen 4

References

1. Majidpoor, J.; Mortezaee, K. Steps in metastasis: An updated review. Med. Oncol. 2021, 38, 3. [CrossRef] [PubMed]
2. Paget, S. The distribution of secondary growths in cancer of the breast. The Lancet 1889, 133, 571–573. [CrossRef]
Wang, Y.; Ma, J.; Shen, H.; Wang, C.; Sun, Y.; Howell, S.B.; Lin, X. Reactive oxygen species promote ovarian cancer progression via LOX pathway.

Chan, C.Y.; Yuen, V.; Wong, C.C. Hypoxia and the Metastatic Niche. In Hypoxia and Cancer Metastasis; Springer: Cham, Switzerland, 2019; pp. 97–112.

Chang, J.; Erler, J. Hypoxia-Mediated Metastasis. Adv. Exp. Med. Biol. 2014, 772, 55–81. [CrossRef]

Araos, J.; Sleeman, J.P.; Garvalov, B.K. The role of hypoxic signalling in metastasis: Towards translating knowledge of basic biology into novel anti-tumour strategies. Clin. Exp. Metastasis 2015, 35, 565–599. [CrossRef]

Chin, A.R.; Wang, S.E. Cancer Tills the Premetastatic Field: Mechanistic Basis and Clinical Implications. Clin. Cancer Res. 2016, 22, 3725–3733. [CrossRef]

Chen, J.; Michie, S.; Seve, M. Le sécrétome: Définitions et intérêt biomédical. La Rev. Médecine Interne 2008, 29, 606–608. [CrossRef]

Liu, Y.; Ciotti, G.E.; Eisenger-Mathason, T.S. Hypoxia and the Tumor Secretome. In Hypoxia and Cancer Metastasis; Springer: Cham, Switzerland, 2019; pp. 57–69.

Wong, C.C.-L.; Tse, A.P.; Huang, Y.-P.; Chiu, D.K.-C.; Lai, R.K.-H.; Au, S.L.-K.; Kai, A.K.-L.; Lee, J.M.-F.; et al. Lysyl oxidase-like 2 is critical to tumor microenvironment and metastatic niche formation in hepatocellular carcinoma. Hepatol. 2014, 60, 1645–1658. [CrossRef]

Erler, J.T.; Bennewith, K.L.; Niclou, M.; Dornhöfer, N.; Kong, C.; Le, Q.-T.; Chi, J.-T.A.; Jeffrey, S.S.; Giaccia, A.J. Lysyl oxidase is essential for hypoxia-induced metastasis. Nature 2006, 440, 1222–1226. [CrossRef]

Sion, A.M.; Figg, W.D. Lysyl oxidase (LOX) and hypoxia-induced metastases. Cancer Biol. Ther. 2006, 5, 909–911. [CrossRef]

Cox, T.R.; Garland, A.; Erler, J.T. Lysyl Oxidase, a Targetable Secreted Molecule Involved in Cancer Metastasis. Cancer Res. 2016, 76, 188–192. [CrossRef]

Joshi, H.P.; Subramanian, I.V.; Schnettler, E.K.; Ghosh, G.; Rupaimoole, R.; Evans, C.; Saluja, M.; Jing, Y.; Cristina, I.; Roy, S.; et al. Dynamin 2 along with microRNA-199a reciprocally regulate hypoxia-inducible factors and ovarian cancer metastasis. Proc. Natl. Acad. Sci. USA 2014, 111, 5331–5336. [CrossRef]

Ji, F.; Wang, Y.; Qiu, L.; Li, S.; Zhu, J.; Liang, Z.; Wan, Y.; Di, W. Hypoxia inducible factor 1α-mediated LOX expression correlates with migration and invasion in epithelial ovarian cancer. Int. J. Oncol. 2013, 42, 1578–1588. [CrossRef]

Han, Y.-L.; Chen, L.; Qin, R.; Wang, G.-Q.; Lin, X.-H.; Dai, G.-H. Lysyl oxidase and hypoxia-inducible factor 1α: Biomarkers of gastric cancer. World J. Gastroenterol. 2019, 25, 1828–1839. [CrossRef]

Umezaki, N.; Nakagawa, S.; Yamashita, Y.; Kitano, Y.; Arima, K.; Miyata, T.; Hiyoshi, Y.; Okabe, H.; Nitta, H.; Hayashi, H.; et al. Lysyl oxidase induces epithelial-mesenchymal transition and predicts intrahepatic metastasis of hepatocellular carcinoma. Cancer Sci. 2019, 110, 2033–2043. [CrossRef]

Dar, Z.; Liu, T.; Liu, G.; Deng, Z.; Yu, P.; Wang, B.; Cen, B.; Guo, L.; Zhang, J. Identification of Clinical and Tumor Microenvironment Characteristics of Hypoxia-Related Risk Signature in Lung Adenocarcinoma. Front. Mol. Biol. 2021, 8, 757421. [CrossRef]

Wong, C.C.-L.; Zhang, H.; Gilkes, D.M.; Chen, J.; Wei, H.; Chaturvedi, P.; Hubbi, M.E.; Semenza, G.L. Inhibitors of hypoxia-inducible factor block breast cancer metastatic niche formation and lung metastasis. J. Mol. Med. 2012, 90, 803–815. [CrossRef]

Urooj, T.; Wasim, B.; Mushtaq, S.; Shah, S.N.N.; Shah, M. Cancer Cell-derived Secretory Factors in Breast Cancer-associated Lung Metastasis: Their Role and Future Prospects. Curr. Cancer Drug Targets 2020, 20, 168–186. [CrossRef]

Wang, Y.; Ma, J.; Shen, H.; Wang, C.; Sun, Y.; Howell, S.B.; Lin, X. Reactive oxygen species promote ovarian cancer progression via the HIF-1α/LOX/E-cadherin pathway. Oncol. Rep. 2014, 32, 2150–2158. [CrossRef]

Joo, Y.N.; Jin, H.; Eun, S.Y.; Park, S.W.; Chang, K.C.; Kim, H.J. P2Y2R activation by nucleotides released from the highly metastatic breast cancer cell contributes to pre-metastatic niche formation by mediating lysyl oxidase secretion, collagen crosslinking, and monocyte recruitment. Oncotarget 2014, 5, 9322–9334. [CrossRef] [PubMed]

Wang, V.; Davis, D.A.; Yarchan, R. Identification of functional hypoxia inducible factor response elements in the human lysyl oxidase gene promoter. Biochem. Biophys. Res. Commun. 2017, 480, 480–485. [CrossRef] [PubMed]

Erler, J.T.; Bennewith, K.L.; Cox, T.R.; Lang, G.; Bird, D.; Koong, A.; Le, Q.-T.; Giaccia, A.J. Hypoxia-Induced Lysyl Oxidase Is a Critical Mediator of Bone Marrow Cell Recruitment to form the Premetastatic Niche. Cancer Cell 2009, 15, 35–44. [CrossRef] [PubMed]

Semenza, G.L. Cancer–stromal cell interactions mediated by hypoxia-inducible factors promote angiogenesis, lymphangiogenesis, and metastasis. Oncogene 2013, 32, 4057–4063. [CrossRef] [PubMed]

Natarajan, S.; Foreman, K.M.; Soriano, M.J.; Rossen, N.S.; Shehade, H.; Fregoso, D.R.; Eggold, J.T.; Krishnan, V.; Dorigo, O.; Krieg, A.J.; et al. Collagen Remodeling in the Hypoxic Tumor-Mesothelial Niche Promotes Ovarian Cancer Metastasis. Cancer Res. 2019, 79, 2271–2284. [CrossRef]

Wong, C.C.-L.; Gilkes, D.M.; Zhang, H.; Chen, J.; Wei, H.; Chaturvedi, P.; Fraley, S.I.; Wong, C.-M.; Khoo, U.-S.; Ng, L.O.-L.; et al. Hypoxia-inducible factor 1 is a master regulator of breast cancer metastatic niche formation. Proc. Natl. Acad. Sci. USA 2011, 108, 16369–16374. [CrossRef]

Baker, A.-M.; Bird, D.; Lang, G.; Cox, T.R.; Erler, J.T. Lysyl oxidase enzymatic function increases stiffness to drive colorectal cancer progression through FAK. Oncogene 2013, 32, 1863–1868. [CrossRef]
30. Zhu, G.; Wang, L.; Meng, W.; Lu, S.; Cao, B.; Liang, X.; He, C.; Hao, Y.; Du, X.; Wang, X.; et al. LOXL2-enriched small extracellular vesicles mediate hypoxia-induced premetastatic niche and indicates poor outcome of head and neck cancer. *Theranostics* **2021**, *11*, 9198–9216. [CrossRef]

31. Todd, V.M.; Johnson, R.W. Hypoxia in bone metastasis and osteolysis. *Cancer Lett.* **2020**, *489*, 144–154. [CrossRef]

32. Li, Q.; Zhu, C.-C.; Ni, B.; Zhang, Z.-Z.; Jiang, S.-H.; Hu, L.-P.; Wang, X.; Zhang, X.-X.; Huang, P.-Q.; Yang, Q.; et al. Lysyl oxidase promotes liver metastasis of gastric cancer via facilitating the reciprocal interactions between tumor cells and cancer associated fibroblasts. *BioMedicine* **2019**, *9*, 157–171. [CrossRef]

33. Yang, Y.-L.; Tsai, M.-C.; Chang, Y.-H.; Wang, C.-C.; Chu, P.-Y.; Lin, H.-Y.; Huang, Y.-H. MIR29A Impedes Metastatic Behaviors in Hepatocellular Carcinoma via Targeting LOX, LOXL2, and VEGFA. *Int. J. Mol. Sci.* **2021**, *22*, 6001. [CrossRef]

34. Miller, B.W.; Morton, J.P.; Fines, M.; Saturno, G.; Jamieson, N.B.; McGhee, E.; Timpson, P.; Leach, J.; McGarry, L.; Shanks, E.; et al. Targeting the LOX/hypoxia axis reverses many of the features that make pancreatic cancer deadly: Inhibition of LOX abrogates metastasis and enhances drug efficacy. *EMBO Mol. Med.* **2015**, *7*, 1063–1076. [CrossRef]

35. Santhanam, A.N.; Baker, A.R.; Hegamyer, G.; Kirschmann, D.A.; Colburn, N.H. Pdcd4 repression of lysyl oxidase inhibits hypoxia-induced breast cancer cell invasion. *Oncogene* **2010**, *29*, 3921–3932. [CrossRef]

36. Miller, B.W.; Morton, J.P.; Fines, M.; Saturno, G.; Jamieson, N.B.; McGhee, E.; Timpson, P.; Leach, J.; McGarry, L.; Shanks, E.; et al. Targeting the LOX/hypoxia axis reverses many of the features that make pancreatic cancer deadly: Inhibition of LOX abrogates metastasis and enhances drug efficacy. *EMBO Mol. Med.* **2015**, *7*, 1063–1076. [CrossRef]

37. Salvador, F.; Martin, A.; López-Menéndez, C.; Moreno-Bueno, G.; Santos, V.; Vázquez-Naharro, A.; Santamaria, P.G.; Morales, S.; Dubus, P.R.; Muinelo-Romay, L.; et al. Lysyl Oxidase–like Protein LOXL2 Promotes Lung Metastasis of Breast Cancer. *Cancer Res.* **2017**, *77*, 5846–5859. [CrossRef]

38. Roberts, E.; Cossigny, D.A.F.; Quan, G.M.Y. The Role of Vascular Endothelial Growth Factor in Metastatic Prostate Cancer to the Skeleton. *Prostate Cancer* **2013**, *2013*, 418340. [CrossRef]

39. Wakisaka, N.; Hasegawa, Y.; Yoshimoto, S.; Miura, K.; Shiotani, A.; Yokoyama, J.; Sugawara, M.; Moriyama-Kita, M.; Endo, K.; Yoshizaki, T. Primary Tumor-Secreted Lympangioendothelial Factors Induce Pre-Metastatic Lymphvascular Niche Formation at Sentinel Lymph Nodes in Oral Squamous Cell Carcinoma. *PLoS ONE* **2015**, *10*, e0144056. [CrossRef]

40. Carano, R.A.; Filvaroff, E.H. Angiogenesis and bone repair. *Drug Discov. Today* **2003**, *8*, 980–989. [CrossRef]

41. Dai, J.; Kitagawa, Y.; Zhang, J.; Yao, Z.; Mizokami, A.; Cheng, S.; Nör, J.; McCauley, L.K.; Taichman, R.S.; Keller, E.T. Vascular Endothelial Growth Factor Contributes to the Prostate Cancer-Induced Osteoblast Differentiation Mediated by Bone Morphogenetic Protein. *Cancer Res.* **2004**, *64*, 994–999. [CrossRef]

42. Kitagawa, Y.; Dai, J.; Zhang, J.; Keller, J.M.; Nor, J.; Yao, Z.; Keller, E.T. Vascular Endothelial Growth Factor Contributes to Prostate Cancer–Mediated Osteoblastic Activity. *Cancer Res.* **2005**, *65*, 10921–10929. [CrossRef]

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

44. Van Der Pluijm, G.; Sijmons, B.; Vloedgraven, H.; Decker, M.; Papapoulos, S.; Löwik, C. Monitoring Metastatic Behavior of Human Tumor Cells in Mice with Species-Specific Polymerease Chain Reaction: Elevated Expression of Angiogenesis and Bone Resorption Stimulators by Breast Cancer in Bone Metastases. *J. Bone Miner. Res.* **2001**, *16*, 1077–1091. [CrossRef] [PubMed]

45. Weilbaecher, K.N.; Guise, T.A.; McCauley, L.K. Cancer to bone: A fatal attraction. *Nat. Rev. Cancer* **2011**, *11*, 411–425. [CrossRef] [PubMed]

46. Hiratsuka, S.; Watanabe, A.; Sakurai, Y.; Akashi-Takamura, S.; Ishibashi, S.; Miyake, K.; Shibuya, M.; Akira, S.; Aburatani, H.; Maru, Y. The S100A8–serum amyloid A3–TLR4 paracrine cascade establishes a pre-metastatic phase. *Nat. Cell Biol.* **2013**, *15*, 1369–1375. [CrossRef]

47. Srikrishna, G. S100A8 and S100A9: New Insights into Their Roles in Malignancy. *J. Innate Immun.* **2012**, *4*, 31–40. [CrossRef] [PubMed]

48. Xu, W.W.; Li, B.; Guan, X.Y.; Chung, S.K.; Wang, Y.; Yip, Y.L.; Law, S.Y.K.; Chan, K.T.; Lee, N.P.Y.; Chan, K.W.; et al. Cancer cell-secreted IGF2 instigates fibroblasts and bone marrow-derived vascular progenitor cells to promote cancer progression. *Nat. Commun.* **2017**, *8*, 14399. [CrossRef]

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

50. Chang, W.; Tsai, Y.; Tsai, Y.; Wu, C.; Chang, K.; Lien, C.; Hung, J.; Hsu, Y.; Kuo, P. Differential expression profiles of the transcriptome in bone marrow-derived cells in lung cancer revealed by next generation sequencing and bioinformatics. *OncoLett.* **2019**, *17*, 4341–4350. [CrossRef]

51. Sterling, J.A.; Edwards, J.R.; Martin, T.J.; Mundy, G.R. Advances in the biology of bone metastasis: How the skeleton affects tumor behavior. *Bone* **2011**, *48*, 6–15. [CrossRef]

52. Chen, J.; De, S.; Brainard, J.; Byzova, T.V. Metastatic Properties of Prostate Cancer Cells are Controlled by VEGF. *Cell Commun. Adhes.* **2004**, *11*, 1–11. [CrossRef]

53. Liu, S.; Jiang, M.; Zhao, Q.; Li, S.; Peng, Y.; Zhang, P.; Han, M. Vascular endothelial growth factor plays a critical role in the formation of the pre-metastatic niche via prostaglandin E2. *Oncol. Rep.* **2014**, *27*, 2477–2484. [CrossRef]

54. Hiratsuka, S.; Watanabe, A.; Aburatani, H.; Maru, Y. Tumour-mediated upregulation of chemotactants and recruitment of myeloid cells predetermines lung metastasis. *Nat. Cell Biol.* **2006**, *8*, 1369–1375. [CrossRef]
55. Efferth, T.; Leber, M.F. Molecular principles of cancer invasion and metastasis (Review). Int. J. Oncol. 2009, 34, 881–895. [CrossRef]

56. Li, R.; Qi, Y.; Jiang, M.; Zhang, T.; Wang, H.; Wang, L.; Han, M. Primary tumor-secreted VEGF induces vascular hyperpermeability in premetastatic lung via the occludin phosphorylation/ubiquitination pathway. Mol. Carcinog. 2019, 58, 2316–2326. [CrossRef]

57. Marvin, D.L.; Heijbroe, R.; ten Dijke, P.; Ritsma, L. TGF-β signaling in liver metastasis. Clin. Transl. Med. 2020, 10, e160. [CrossRef]

58. Ayabe, H.; Anada, T.; Kamaya, T.; Sato, T.; Kimura, M.; Yoshizawa, E.; Kikuchi, S.; Ueno, Y.; Sekine, K.; Camp, J.G.; et al. Optimal Hypoxia Regulates Human iPSC-Derived Liver Bud Differentiation through Intercellular TGFβ Signaling. Stem. Cell Reports 2018, 11, 306–316. [CrossRef]

59. Mallikarjuna, P.; Zhou, Y.; Landström, Y. The Synergistic Cooperation between TGF-β and Hypoxia in Cancer and Fibrosis. Biomolecules 2022, 12, 635. [CrossRef]

60. Ashraf, M.A.B.; Zahid, A.; Ashraf, S.; Waqar, S.; Iqbal, S.; Malik, A. Implication of Prophetic Variables and their Impulsive Interplay in CA Prostate Patients Experiencing Osteo-Metastasis. Anticancer. Agents Med. Chem. 2020, 20, 2106–2113. [CrossRef]

61. Rynne-Vidal, A.; Au-Youeng, C.L.; Jiménez-Heffernan, J.A.; Pérez-Lozano, M.L.; Creames-Jimeno, L.; Bárcena, C.; Cristóbal-García, I.; Fernández-Chacón, C.; Yeung, T.L.; Mok, S.C.; et al. Mesothelial-to-mesenchymal transition as a possible therapeutic target in peritoneal metastasis of ovarian cancer. J. Pathol. 2017, 242, 140–151. [CrossRef]

62. Zhu, M.; Zhang, N.; Ma, J.; He, S. Integration of exosomal miR-106a and mesothelial cells facilitates gastric cancer peritoneal dissemination. Cell. Signal. 2022, 91, 110230. [CrossRef]

63. Mazumdar, A.; Urdeinez, J.; Boro, A.; Migliafaccia, J.; Arlt, M.J.E.; Muff, R.; Fuchs, B.; Snedeger, J.G.; Gvozdenovic, A. Osteosarcoma-Derived Extracellular Vesicles Induce Lung Fibroblast Reprogramming. Int. J. Mol. Sci. 2020, 21, 5451. [CrossRef] [PubMed]

64. Li, B.; Xia, Y.; Lv, J.; Wang, W.; Xuan, Z.; Chen, C.; Jiang, T.; Fang, L.; Wang, L.; Li, Z.; et al. miR-151a-3p-rich small extracellular vesicles derived from gastric canceraccelerate liver metastasis via initiating a hepatic stemness-enhancing niche. Oncogene 2021, 40, 6180–6194. [CrossRef] [PubMed]

65. Yan, H.H.; Jiang, J.; Pang, Y.; Achyut, B.R.; Lizardo, M.; Liang, X.; Hunter, K.; Khanna, C.; Hollander, C.; Yang, L. CCL9 Induced by TGFβ Signaling in Myeloid Cells Enhances Tumor Cell Survival in the Premetastatic Organ. Cancer Res. 2015, 75, 5283–5298. [CrossRef] [PubMed]

66. Battle, E.; Massagué, J. Transforming Growth Factor-β Signaling in Immunity and Cancer. Immunity 2019, 50, 924–940. [CrossRef] [PubMed]

67. Tauriello, D.V.F.; Palomo-Ponce, S.; Stork, D.; Berenguer-Llergo, A.; Badia-Ramentol, J.; Iglesias, M.; Sevillano, M.; Ibiza, S.; Cañellas, A.; Hernando-Mombiola, X.; et al. TGFβ drives immune evasion in genetically reconstituted colon cancer metastases. Nature 2018, 554, 538–543. [CrossRef]

68. Costa-Silva, B.; Aiello, N.M.; Ocean, A.J.; Singh, S.; Zhang, H.; Thakur, B.K.; Becker, A.; Hoshino, A.; Mark, M.T.; Molina, H.; et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. Nat. Cell Biol. 2015, 17, 816–826. [CrossRef]

69. Zhou, W.; Ke, S.Q.; Huang, Z.; Flavahan, W.; Fang, X.; Paul, J.; Wu, L.; Sloan, A.E.; McLendon, R.E.; Li, X.; et al. Periostatin secreted by glioblastoma stem cells recruits M2 tumour-associated macrophages and promotes malignant growth. Nat. Cell Biol. 2015, 17, 170–182. [CrossRef]

70. Kerr, B.A.; Harris, K.S.; Shi, L.; Willey, J.S.; Soto-Pantoja, D.R.; Byzova, T. V Platelet TSP-1 controls prostate cancer-induced osteoclast differentiation and bone marrow-derived cell mobilization through TGFβ-1. Am. J. Clin. Exp. Urol. 2021, 9, 18–31.

71. Tomar, J.S.; Shen, J. Characterization of Carbonic Anhydrase In Vivo Using Magnetic Resonance Spectroscopy. Int. J. Mol. Sci. 2020, 21, 2442. [CrossRef]

72. Nolly, M.B.; Vargas, L.A.; Correa, M.V.; Lofeudo, J.M.; Pinilla, A.O.; Rueda, J.O.V.; Guerrero-Gimenez, M.E.; Swenson, E.R.; Damiani, M.T.; Alvarez, B.V. Carbonic anhydrase IX and hypoxia-inducible factor 1 attenuate cardiac dysfunction after myocardial infarction. Pflügers Arch. Eur. J. Physiol. 2021, 473, 1273–1285. [CrossRef]

73. McDonald, P.C.; Dedhar, S. Carbonic Anhydrase IX (CAIX) as a Mediator of Hypoxia-Induced Stress Response in Cancer Cells; Springer: Dordrecht, The Netherlands, 2014; pp. 255–269.

74. Ong, C.H.C.; Lee, D.Y.; Lee, B.; Li, H.; Lim, J.C.T.; Lim, J.X.; Yeong, J.P.S.; Lau, H.Y.; Thike, A.A.; Tan, P.H.; et al. Hypoxia-regulated carbonic anhydrase IX (CAIX) protein is an independent prognostic indicator in triple negative breast cancer. Breast Cancer Res. 2022, 24, 38. [CrossRef]

75. Hedlund, E.-M.E.; McDonald, P.C.; Nemirovsky, O.; Awey, S.; Jensen, L.D.E.; Dedhar, S. Harnessing Induced Essentiality: Targeting Carbonic Anhydrase IX and Angiogenesis Reduces Lung Metastasis of Triple Negative Breast Cancer Xenografts. Cancers 2019, 11, 1002. [CrossRef]

76. Schmidt, J.; Oppermann, E.; Blaheta, R.A.; Schreckenbach, T.; Lunger, I.; Rieger, M.A.; Behstein, W.O.; Holzer, K.; Malkomes, P. Carbonic-anhydrase IX expression is increased in thyroid cancer tissue and represents a potential therapeutic target to eradicate thyroid tumor-initiating cells. Mol. Cell. Endocrinol. 2021, 535, 111382. [CrossRef]

77. Kalinin, S.; Malkova, A.; Sharonova, T.; Sharovyko, V.; Bunev, A.; Supuran, C.T.; Krasavin, M. Carbonic Anhydrase IX Inhibitors as Candidates for Combination Therapy of Solid Tumors. Int. J. Mol. Sci. 2021, 22, 13405. [CrossRef]

78. Hsin, M.-C.; Hsieh, Y.-H.; Hsiao, Y.-H.; Chen, P.-N.; Wang, P.-H.; Yang, S.-F. Carbonic Anhydrase IX Promotes Human Cervical Cancer Cell Motility by Regulating PKFB4 Expression. Cancers 2021, 13, 1174. [CrossRef]

79. Zhang, X.; Liu, X.; Cui, W.; Zhang, R.; Liu, Y.; Li, Y.; Hao, J. Sohlh2 alleviates malignancy of EOC cells under hypoxia via inhibiting the HIF1α/CA9 signaling pathway. Biol. Chem. 2020, 401, 263–271. [CrossRef]
80. Pastorekova, S.; Gillies, R.J. The role of carbonic anhydrase IX in cancer development: Links to hypoxia, acidosis, and beyond. *Cancer Metastasis Rev.* **2019**, *38*, 65–77. [CrossRef]

81. Swayampakula, M.; McDonald, P.C.; Vallejo, M.; Coyaud, E.; Chafe, S.C.; Westerback, A.; Venkateswaran, G.; Shankar, J.; Gao, G.; Laurent, E.M.N.; et al. The interactome of metabolic enzyme carbonic anhydrase IX reveals novel roles in tumor cell migration and invadopodia/MMP14-mediated invasion. *Oncogene* **2017**, *36*, 6244–6261. [CrossRef]

82. Yang, J.-S.; Lin, C.-W.; Hsieh, Y.-H.; Chien, M.-H.; Chuang, C.-Y.; Yang, S.-F. Overexpression of carbonic anhydrase IX induces cell motility by activating matrix metalloproteinase-9 in human oral squamous cell carcinoma cells. *Oncotarget* **2017**, *8*, 83088–83099. [CrossRef]

83. Chafe, S.C.; Lou, Y.; Sceneay, J.; Vallejo, M.; Hamilton, M.J.; McDonald, P.C.; Bennewith, K.L.; Möller, A.; Dedhar, S. Carbonic Anhydrase IX Promotes Myeloid-Derived Suppressor Cell Mobilization and Establishment of a Metastatic Niche by Stimulating G-CSF Production. *Cancer Res.* **2015**, *75*, 996–1008. [CrossRef]

84. Kowanetz, M.; Wu, D.; Lee, J.; Tan, M.; Hagenbeek, T.; Qu, X.; Yu, L.; Ross, J.; Korosisaari, N.; Cao, T.; et al. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 21248–21255. [CrossRef] [PubMed]

85. Sceneay, J.; Chow, M.T.; Chen, A.; Halse, H.M.; Wong, C.S.F.; Andrews, D.M.; Sloan, E.K.; Parker, B.S.; Bowtell, D.D.; Smyth, M.J.; et al. Primary Tumor Hypoxia Recruits CD11b+/Ly6Cmed/Ly6G+ Immune Suppressor Cells and Compromises NK Cell Cytotoxicity in the Premetastatic Niche. *Cancer Res.* **2012**, *72*, 3906–3911. [PubMed]

86. Horie, K.; Kawakami, K.; Fujita, Y.; Sugaya, M.; Kameyama, K.; Mizutani, K.; Deguchi, T.; Ito, M. Exosomes expressing carbonic anhydrase IX promote angiogenesis. *Biochem. Biophys. Res. Commun.* **2017**, *492*, 356–361. [CrossRef] [PubMed]

87. Ledaki, I.; McIntyre, A.; Wigfield, S.; Buffa, F.; McGowan, S.; Baban, D.; Li, J.; Harris, A.L. Carbonic anhydrase IX induction defines a heterogeneous cancer cell response to hypoxia and mediates stem cell-like properties and sensitivity to HDAC inhibition. *Oncotarget* **2015**, *6*, 19413–19427. [CrossRef] [PubMed]

88. Marie-Egyptienne, D.T.; Chaudary, N.; Kalliomäki, T.; Hedley, D.W.; Hill, R.P. Cancer initiating-cells are enriched in the CA9 positive fraction of primary cervix cancer xenografts. *Oncotarget* **2017**, *8*, 1392–1404. [CrossRef]

89. Tomita, T.; Sakurai, Y.; Ishibashi, S.; Maru, Y. Imbalance of Clara cell-mediated homeostatic inflammation is involved in lung metastasis. *Oncogene* **2011**, *30*, 3429–3439. [CrossRef] [PubMed]

90. Lukandin, E.; Sleeman, J.P. Building the niche: The role of the S100 proteins in metastatic growth. *Int. Immunopharmacol.* **2011**, *11*, 57–62. [CrossRef]

91. Grivennikov, S.I.; Karin, M. Inflammatory cytokines in cancer: Tumour necrosis factor and interleukin 6 take the stage. *Curr. Opin. Immunol.* **2011**, *23*, 70–75. [CrossRef] [PubMed]

92. Gao, D.; Joshi, N.; Choi, H.; Ryu, S.; Hahn, M.; Catena, R.; Sadik, H.; Argani, P.; Wagner, P.; Vahdat, L.T.; et al. Myeloid Progenitor Cells in the Premetastatic Lung Promote Metastases by Inducing Mesenchymal to Epithelial Transition. *Cancer Res.* **2012**, *72*, 1384–1394. [CrossRef]

93. Yan, H.H.; Pickup, M.; Pang, Y.; Gorska, A.E.; Li, Z.; Chytil, A.; Geng, Y.; Gray, J.W.; Moses, H.L.; Yang, L. Gr-1+CD11b+ Myeloid Cells Tip the Balance of Immune Protection to Tumor Promotion in the Premetastatic Lung. *Cancer Res.* **2010**, *70*, 6139–6149. [CrossRef]

94. Jing, B.; Wang, T.; Sun, B.; Xu, J.; Xu, D.; Liao, Y.; Song, H.; Guo, W.; Li, K.; Hu, M.; et al. IL6/STAT3 Signaling Orchestrates Premetastatic Niche Formation and Immunosuppressive Traits in Lung. *Cancer Res.* **2020**, *80*, 784–797. [CrossRef]

95. Lee, E.; Fertig, E.J.; Jin, K.; Sukumar, S.; Pandey, N.B.; Popel, A.S. Breast cancer cells condition lymphatic endothelial cells within pre-metastatic niches to promote metastasis. *Nat. Commun.* **2014**, *5*, 4715. [CrossRef]

96. Bellido, T.; Borba, V.Z.C.; Roberson, P.; Manolagas, S.C. Activation of the Janus Kinase/STAT (Signal Transducer and Activator of Transcription) Signal Transduction Pathway by Interleukin-6-Type Cytokines Promotes Osteoblast Differentiation. *Endocrinology* **1997**, *138*, 3666–3676. [CrossRef]

97. Sottnik, J.L.; Keller, E.T. Understanding and Targeting Osteoclast Activity in Prostate Cancer Bone Metastases. *Curr. Mol. Med.* **2013**, *13*, 626–639. [CrossRef]

98. Yin, J.J.; Pollock, C.B.; Kelly, K. Mechanisms of cancer metastasis to the bone. *Cell Res.* **2005**, *15*, 57–62. [CrossRef]

99. Ibrahim, T.; Flamini, E.; Mercatali, L.; Sacanna, E.; Serra, P.; Amadori, D. Pathogenesis of osteoblastic bone metastases from prostate cancer. *Cancer 2010*, *116*, 406–418. [CrossRef]

100. Rucci, N.; Angelucci, A. Prostate Cancer and Bone: The Elective Affinities. *Biomed Res. Int.* **2014**, *2014*, 167035. [CrossRef]

101. Mizutani, K.; Sud, S.; Pienta, K.J. Prostate cancer promotes CD11b positive cells to differentiate into osteoclasts. *J. Cell. Biochem.* **2009**, *106*, 563–569. [CrossRef]

102. Kim, S.W.; Kim, J.S.; Papadopoulos, J.; Choi, H.J.; He, J.; Maya, M.; Langlely, R.R.; Fan, D.; Fidler, I.J.; Kim, S.-J. Consistent interactions between tumor IL-6 and macrophage TNF-α enhance the growth of human prostate cancer cells in the bone of nude mouse. *Int. Immunopharmacol.* **2011**, *11*, 862–872. [CrossRef]

103. Jiang, J.; Wang, G.-Z.; Wang, Y.; Huang, H.-Z.; Li, W.-T.; Qu, X.-D. Hypoxia-induced HMGBl expression of HCC promotes tumor invasiveness and metastasis via regulating macrophage-derived IL-6. *Exp. Cell Res.* **2018**, *367*, 81–88. [CrossRef]

104. Wang, X.; Lee, S.O.; Xia, S.; Jiang, Q.; Luo, J.; Li, L.; Yeh, S.; Chang, C. Endothelial Cells Enhance Prostate Cancer Metastasis via IL-6→Androgen Receptor→TGF-β→MMP-9 Signals. *Mol. Cancer Ther.* **2013**, *12*, 1026–1037. [CrossRef] [PubMed]
105. Manisterski, M.; Golan, M.; Amir, S.; Weisman, Y.; Mabjeesh, N.J. Hypoxia induces PTHrP gene transcription in human cancer cells through the HIF-2α. Cell Cycle 2010, 9, 3723–3729. [CrossRef] [PubMed]

106. Rankin, E.B.; Giaccia, A.J. Hypoxic control of metastasis. Science 2016, 352, 175–180. [CrossRef] [PubMed]

107. Guo, F.; Wang, Y.; Liu, J.; Mok, S.C.; Xue, F.; Zhang, W. CXCL12/CXCR4: A symbiotic bridge linking cancer cells and their stromal neighbors in oncogenic communication networks. Oncogene 2016, 35, 816–826. [CrossRef] [PubMed]

108. Jin, F.; Brockmeier, U.; Otterbach, F.; Metzen, E. New Insight into the SDF-1/CXCR4 Axis in a Breast Carcinoma Model: Hypoxia-Induced Endothelial SDF-1 and Tumor Cell CXCR4 Are Required for Tumor Cell Infiltration. Mol. Cancer Res. 2012, 10, 1021–1031. [CrossRef]

109. Dai, J.; Escara-Wilke, J.; Keller, J.M.; Jung, Y.; Taichman, R.S.; Pienta, K.J.; Keller, E.T. Primary prostate cancer educates bone stroma through exosomal pyruvate kinase M2 to promote bone metastasis. J. Exp. Med. 2019, 216, 2883–2899. [CrossRef]

110. Greer, S.N.; Metcalfe, J.L.; Wang, Y.; Ohh, M. The updated biology of hypoxia-inducible factor. EMBO J. 2012, 31, 2448–2460. [CrossRef]

111. Oskarsson, T.; Acharyya, S.; Zhang, X.H.-F.; Vanharanta, S.; Tavazoie, S.F.; Morris, P.G.; Downey, R.J.; Manova-Todorova, K.; Brogi, E.; Massagué, J. Breast cancer cells produce tenascin C as a metastatic niche component to colonize the lungs. Nat. Med. 2011, 17, 867–874. [CrossRef]

112. Lee, J.; Yoon, Y.; Lee, S. Hypoxic Preconditioning Promotes the Bioactivities of Mesenchymal Stem Cells via the HIF-1α-GRP78-Akt Axis. Int. J. Mol. Sci. 2017, 18, 1320. [CrossRef]

113. Raposo, G.; Nijman, H.W.; Stoorvogel, W.; Liejendekker, R.; Melief, C.J.; Geuze, H.J. B lymphocytes secrete membrane-Bound and Exosomal Metastasis-Associated C4.4A Promotes Migration and Invasion of Ovarian Carcinoma Cells. Oncol. Lett. 2014, 8, 1770–1780. [CrossRef]

114. Kahlert, C.; Kalluri, R. Exosomes in tumor microenvironment influence cancer progression and metastasis. J. Mol. Med. 2013, 91, 431–437. [CrossRef]

115. Chen, L.; Zheng, H.; Yu, X.; Liu, L.; Li, H.; Zhu, H.; Zhang, Z.; Lei, P.; Shen, G. Tumor-Secreted GRP78 Promotes the Establishment of a Pre-metastatic Niche in the Liver Microenvironment. Front. Immunol. 2020, 11, 584458. [CrossRef]

116. Li, L.; Chen, C.; Wang, S.; Wang, Z.; Jiang, J.; Wang, W.; Li, X.; Chen, J.; Liu, K.; Li, C.; et al. Exosomes Derived from Hypoxic Oral Squamous Cell Carcinoma Cells Deliver miR-21 to Normoxic Cells to Elicit a Prometastatic Phenotype. Cancer Res. 2016, 76, 1770–1780. [CrossRef]

117. Xue, M.; Chen, W.; Xiang, A.; Wang, R.; Chen, H.; Pan, J.; Pang, H.; An, H.; Wang, X.; Hou, H.; et al. Hypoxia exosomes facilitate bladder tumor growth and development through transferring long non-coding RNA-UCAI. Mol. Cancer 2017, 16, 143. [CrossRef]

118. Wang, Y.; Yi, J.; Chen, X.; Zhang, Y.; Xu, M.; Yang, Z. The regulation of cancer cell migration by lung cancer cell-derived exosomes through TGF-β and IL-10. Oncol. Lett. 2016, 11, 1527–1530. [CrossRef] [PubMed]

119. Ramteke, A.; Ting, H.; Agarwal, C.; Mateen, S.; Somasagara, R.; Hussain, A.; Graner, M.; Frederick, B.; Agarwal, R.; Deep, G. Exosomes secreted under hypoxia enhance invasiveness and stemness of prostate cancer cells by targeting adherens junction molecules. Mol. Carcinog. 2015, 54, 554–565. [CrossRef] [PubMed]

120. Wang, T.; Gilkes, D.M.; Takano, N.; Xiang, L.; Luo, W.; Bishop, C.J.; Chaturvedi, P.; Green, J.J.; Semenza, G.L. Hypoxia-inducible factors and RAB22A mediate formation of microvesicles that stimulate breast cancer invasion and metastasis. Proc. Natl. Acad. Sci. USA 2014, 111, E3234–E3242. [CrossRef] [PubMed]

121. Ngora, H.; Galli, U.M.; Miyazaki, K.; Zöller, M. Membrane-Bound and Exosomal Metastasis-Associated C4.4A Promotes Migration by Associating with the α6β4 Integrin and MT1-MMP. Neoplasia 2012, 14, 95-IN2. [CrossRef] [PubMed]

122. Gutwein, P.; Stoeck, A.; Riedle, S.; Gost, D.; Runz, S.; Condon, T.P.; Marmé, A.; Phong, M.-C.; Linderkamp, O.; Skorokhod, A.; et al. Cleavage of L1 in Exosomes and Apoptotic Membrane Vesicles Released from Ovarian Carcinoma Cells. Clin. Cancer Res. 2005, 11, 2492–2501. [CrossRef] [PubMed]
129. Peinado, H.; Lavotshkin, S.; Lyden, D. The secreted factors responsible for pre-metastatic niche formation: Old sayings and new thoughts. *Semin. Cancer Biol.* 2011, 21, 139–146. [CrossRef]

130. Peinado, H.; Alejó, M.; Lavotshkin, S.; Matei, I.; Costa-Silva, B.; Moreno-Bueno, G.; Hergueta-Redondo, M.; Williams, C.; Garcia-Santos, G.; Ghaajar, C.M.; et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* 2012, 18, 883–891. [CrossRef]

131. Qian, D.; Xie, Y.; Huang, M.; Gu, J. Tumor-derived exosomes in hypoxic microenvironment: Release mechanism, biological function and clinical application. *J. Cancer* 2022, 13, 1685–1694. [CrossRef]

132. Chen, X.; Zhou, J.; Li, X.; Wang, X.; Lin, Y.; Wang, X. Exosomes derived from hypoxic epithelial ovarian cancer cells deliver microRNAs to macrophages and elicit a tumor-promoted phenotype. *Cancer Lett.* 2018, 435, 80–91. [CrossRef]

133. Peinado, H.; Aleksicovic, M.; Lavotshkin, S.; Matei, I.; Costa-Silva, B.; Moreno-Bueno, G.; Hergueta-Redondo, M.; Williams, C.; Garcia-Santos, G.; Ghaajar, C.M.; et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* 2012, 18, 883–891. [CrossRef]

134. Tadokoro, H.; Umezu, T.; Ohyashiki, K.; Hirano, T.; Ohyashiki, J.H. Exosomes Derived from Hypoxic Leukemia Cells Enhance Tube Formation in Endothelial Cells. *J. Pathol.* 2016, 240, 329–340. [CrossRef]

135. Li, L.; Cao, B.; Liang, X.; Lu, S.; Luo, H.; Wang, Z.; Wang, S.; Jiang, J.; Lang, J.; Zhu, G. Microenvironmental oxygen pressure orchestrates an anti- and pro-tumoral γδ T cell equilibrium via tumor-derived exosomes. *Oncogene* 2019, 38, 2830–2843. [CrossRef]

136. Dorayappan, K.D.P.; Wanner, R.; Wallbillich, J.J.; Saini, U.; Zingarelli, R.; Suarez, A.A.; Cohn, D.E.; Selvendiran, K. Hypoxia-induced exosomes contribute to a more aggressive and chemoresistant ovarian cancer phenotype: A novel mechanism linking STAT3/Rab proteins. *Oncogene* 2018, 37, 3806–3821. [CrossRef]

137. Panigrahi, G.K.; Praharaj, P.P.; Peak, T.C.; Long, J.; Singh, R.; Rhim, J.S.; Abd Elmageed, Z.Y.; Deep, G. Hypoxia-induced exosome secretion promotes survival of African-American and Caucasian prostate cancer cells. *Sci. Rep.* 2018, 8, 3853. [CrossRef]

138. Wang, Z.; Jin, N.; Ganguli, S.; Swartz, D.R.; Li, L.; Rhoades, R.A. Rho-Kinase Activation Is Involved in Hypoxia-Induced Pulmonary Vasocostriction. *Am. J. Respir. Cell Mol. Biol.* 2001, 25, 628–635. [CrossRef]

139. Pasquet, J.-M.; Toti, F.; Nurden, A.T.; Dachary-Prigent, J. Procoagulant activity and active calpain in platelet-derived microparticles. *Thromb. Res.* 1996, 82, 509–522. [CrossRef]

140. Novgorodov, S.A.; Guzdon, T.Z. Ceramide and Mitochondria in Ischemia/Reperfusion. *J. Cardiovasc. Pharmacol.* 2009, 53, 198–208. [CrossRef]

141. Meng, W.; Hao, Y.; He, C.; Li, L.; Zhu, G. Exosome-orchestrated hypoxic tumor microenvironment. *Cancer Lett.* 2018, 414, 44–56. [CrossRef]

142. Ludwig, N.; Whiteside, T.L. Potential roles of tumor-derived exosomes in angiogenesis. *Expert Opin. Ther. Targets* 2018, 22, 409–417. [CrossRef]

143. Park, J.E.; Tan, H.S.; Sathe, A.; Lim, S.K.; Sze, S.K. Hypoxic Tumor Cell Orchestrates an Anti- and Pro-tumoral Microenvironment to Enhance Angiogenic and Metastatic Potential by Secretion of Proteins and Exosomes. *Cancer Lett.* 2010, 9, 1085–1099. [CrossRef]

144. Tadokoro, H.; Umezu, T.; Ohhashi, K.; Hirato, T.; Ohhashi, J.H. Exosomes Derived from Hypoxic Leukemia Cells Enhance Tube Formation in Endothelial Cells. *J. Biol. Chem.* 2013, 288, 34343–34351. [PubMed] [CrossRef]

145. Hsu, Y.-L.; Hung, J.-Y.; Chang, W.-A.; Lin, Y.-S.; Pan, Y.-C.; Tsai, P.-H.; Wu, C.-Y.; Kuo, P.L. Hypoxia-induced exosomal miR-23a increases angiogenesis and vascular permeability by targeting prolyl hydroxylase and tight junction protein ZO-1. *Oncogene* 2017, 36, 4929–4942. [CrossRef] [PubMed]

146. Paggetti, J.; Haderer, F.; Seiffert, M.; Janji, B.; Distler, U.; Ammerlaan, W.; Kim, Y.J.; Adam, J.; Lichter, P.; Solary, E.; et al. Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. *Blood* 2015, 126, 1106–1117. [CrossRef] [PubMed]

147. Liu, J.; Wu, S.; Zheng, X.; Zheng, P.; Fu, Y.; Wu, C.; Lu, B.; Ju, J.; Jiang, J. Immune suppressed tumor microenvironment by exosomes derived from gastric cancer cells via modulating immune functions. *Sci. Rep.* 2020, 10, 14749. [CrossRef] [PubMed]

148. Yang, L.; Zhang, W.; Yang, Y.; Zou, T.; Zhang, B.; Xu, Y.; Pang, T.; Hu, Q.; Chen, M.; Wang, L.; et al. Hypoxia-induced miR-214 expression promotes tumour cell proliferation and migration by enhancing the Warburg effect in gastric carcinoma cells. *Cancer Lett.* 2018, 414, 44–56. [CrossRef] [PubMed]

149. Ning, T.; Li, J.; He, Y.; Zhang, H.; Wang, X.; Deng, T.; Liu, R.; Li, H.; Bai, M.; Fan, Q.; et al. Exosomal miR-208b related with aoxilipin resistance promotes Treg expansion in colorectal cancer. *Mol. Ther.* 2021, 29, 2723–2736. [CrossRef]

150. Ye, S.-B.; Zhang, H.; Cai, T.-T.; Liu, Y.-N.; Ni, J.-J.; He, J.; Peng, J.-Y.; Chen, Q.-Y.; Mo, H.-Y.; Jun-Cui; et al. Exosomal miR-24-3p impedes T-cell function by targeting FGF11 and serves as a potential prognostic biomarker for nasopharyngeal carcinoma. *J. Pathol.* 2016, 240, 329–340. [CrossRef]

151. Pae, K.; Cao, B.; Liang, X.; Lu, S.; Luo, H.; Wang, Z.; Wang, S.; Jiang, J.; Lang, J.; Zhu, G. Microenvironmental oxygen pressure orchestrates an anti- and pro-tumoral γδ T cell equilibrium via tumor-derived exosomes. *Oncogene* 2019, 38, 2830–2843. [CrossRef] [PubMed]

152. Rong, L.; Li, R.; Li, S.; Luo, R. Immunosuppression of breast cancer cells mediated by transforming growth factor-β in exosomes from cancer cells. *Oncol. Lett.* 2016, 11, 500–504. [CrossRef]

153. Wang, X.; Luo, G.; Zhang, K.; Cao, J.; Huang, C.; Jiang, T.; Liu, B.; Su, L.; Qiu, Z. Hypoxic Tumor-Derived Exosomal miR-301a Mediates M2 Macrophage Polarization via PTEN/PI3K/Akt to Promote Pancreatic Cancer Metastasis. *Cancer Res.* 2018, 78, 4586–4598. [CrossRef]
Cancers 2022, 14, 5930

179. Acosta, J.C.; O’Loghlen, A.; Banito, A.; Guijarro, M.V.; Augert, A.; Raguz, S.; Fumagalli, M.; Da Costa, M.; Brown, C.; Popov, N.; et al. Chemokine Signaling via the CXCR2 Receptor Reinforces Senescence. Cell 2008, 133, 1006–1018. [CrossRef]  

180. Orjalo, A.V.; Bhaumik, D.; Gengler, B.K.; Scott, G.K.; Campisi, J. Cell surface-bound IL-1α is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. Proc. Natl. Acad. Sci. USA 2009, 106, 17031–17036. [CrossRef]  

181. Bhaumik, D.; Scott, G.K.; Schokrpur, S.; Patil, C.K.; Orjalo, A.V.; Rodier, F.; Lithgow, G.J.; Campisi, J. MicroRNAs miR-146a/b negatively modulate the senescence-associated inflammatory mediators IL-6 and IL-8. Aging 2009, 1, 402–411. [CrossRef]  

182. Chambers, C.R.; Ritchie, S.; Pereira, B.A.; Timpson, P. Overcoming the senescence-associated secretory phenotype (SASP): A complex mechanism of resistance in the treatment of cancer. Mol. Oncol. 2021, 15, 3242–3255. [CrossRef]  

183. Veenstra, J.P.; Bittencourt, L.F.F.; Aird, K.M. The Senescence-Associated Secretory Phenotype in Ovarian Cancer Dissemination. Am. J. Physiol. Physiol. 2022, 323, C125–C132. [CrossRef]  

184. Di, G.; Liu, Y.; Lu, Y.; Liu, J.; Wu, C.; Duan, H.-F. IL-6 Secreted from Senescent Mesenchymal Stem Cells Promotes Proliferation and Migration of Breast Cancer Cells. PLoS ONE 2014, 9, e113572. [CrossRef] [PubMed]  

185. Miao, J.-W.; Liu, L.-J.; Huang, J. Interleukin-6-induced epithelial-mesenchymal transition through signal transducer and activator of transcription 3 in human cervical carcinoma. Int. J. Oncol. 2014, 45, 165–176. [CrossRef] [PubMed]  

186. Goulet, C.R.; Champagne, A.; Bernard, G.; Vandal, D.; Chabaud, S.; Pouliot, F.; Bolduc, S. Cancer-associated fibroblasts induce epithelial–mesenchymal transition of bladder cancer cells through paracrine IL-6 signalling. BMC Cancer 2019, 19, 137. [CrossRef]  

187. Wang, L.; Tang, C.; Cao, H.; Li, K.; Pang, X.; Zhong, L.; Dang, W.; Tang, H.; Huang, Y.; Wei, L.; et al. Activation of IL-8 via PI3K/Akt-dependent pathway is involved in leptomatoid epithelial-mesenchymal transition in human breast cancer cells. Cancer Biol. Ther. 2015, 16, 1220–1230. [CrossRef] [PubMed]  

188. Fisher, D.T.; Appenheimer, M.M.; Evans, S.S. The two faces of IL-6 in the tumor microenvironment. Semin. Immunol. 2014, 26, 38–47. [CrossRef]  

189. Waugh, D.J.J.; Wilson, C. The Interleukin-8 Pathway in Cancer. Clin. Cancer Res. 2008, 14, 6735–6741. [CrossRef]  

190. Tam, I.; Kikuchi, T.; Cardinale, I.; Krueger, J.G. Cell-adhesion-disrupting action of interleukin 6 in human ductal breast carcinoma cells. Proc. Natl. Acad. Sci. USA 1994, 91, 3329–3333. [CrossRef]  

191. Ancrile, B.; Lim, K.-H.; Counter, C.M. Oncogenic Ras-induced secretion of IL6 is required for tumorigenesis. Genes Dev. 2007, 21, 1714–1719. [CrossRef]  

192. du Plessis, M.; Davis, T.; Loos, B.; Pretorius, E.; de Villiers, W.J.S.; Engelbrecht, A.M. Molecular regulation of autophagy in a pro-inflammatory tumour microenvironment: New insight into the role of serum amyloid A. Cytokine Growth Factor Rev. 2021, 59, 71–83. [CrossRef]  

193. Ruggiano, A.; Foresti, O.; Carvalho, P. ER-associated degradation: Protein quality control and beyond. Adv. Drug Deliv. Rev. 2020, 11, 843. [CrossRef]  

194. Cheung, K.J.; Ewald, A.J. A collective route to metastasis: Seeding by tumor cell clusters. Front. Oncol. 2018, 8, 189. [CrossRef]  

195. Paret, C.; Schön, Z.; Szponar, A.; Kovacs, G. Inflammatory Protein Serum Amyloid A1 Marks a Subset of Conventional Renal Cell Carcinomas with Fatal Outcome. Eur. Urol. 2010, 57, 859–866. [CrossRef]  

196. Abouelasrar Salama, S.; De Bondt, M.; De Buck, M.; Berghmans, N.; Proost, P.; Oliveira, V.L.S.; Amaral, F.A.; Gouwy, M.; Van Damme, J.; Struyf, S. Serum Amyloid A1 (SAA1) Revisited: Restricted Leukocyte-Activating Properties of Homogeneous SAA1. Front. Immunol. 2020, 11, 843. [CrossRef]  

197. Haghara, K.; Kagawa, S.; Kishida, Y.; Arimitsu, J. Anti-Cytokine Therapy for AA Amyloidosis. In Amyloidosis; InTech: London, UK, 2013.  

198. Aceto, N.; Bardia, A.; Miyamoto, D.T.; Donaldson, M.C.; Wittner, B.S.; Spencer, J.A.; Yu, M.; Pely, A.; Engstrom, A.; Zhu, H.; et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 2014, 158, 1110–1122. [CrossRef]  

199. Laitala, A.; Erler, J.T. Hypoxic Signalling in Tumour Stroma. Front. Oncol. 2018, 8, 189. [CrossRef]  

200. Casazza, A.; Di Conza, G.; Wenes, M.; Deshoemaecker, S.; Mazzone, M. Tumor stroma: A complexity dictated by the hypoxic tumor microenvironment. Oncogene 2014, 33, 1743–1754. [CrossRef]  

201. Kimura, H.; Reinhardt, D.P. Fibronectin-targeted drug delivery in cancer. Adv. Drug Deliv. Rev. 2016, 97, 101–110. [CrossRef]  

202. Dimri, G.P.; Lee, X.; Basile, G.; Acosta, M.; Scott, G.; Roskelley, C.; Medrano, E.E.; Linskens, M.; Rubelj, I.; Pereira-Smith, O. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. USA 1999, 96, 9363–9367. [CrossRef] [PubMed]  

203. Lin, T.-C.; Yang, C.-H.; Cheng, L.-H.; Chang, W.-T.; Lin, Y.-R.; Cheng, H.-C. Fibronectin in Cancer: Friend or Foe. Cells 2019, 8, 27. [CrossRef]  

204. Raught, B.; Persson, A.; Persson, P. ER-associated degradation: Protein quality control and beyond. J. Cell Biol. 2014, 204, 869–879. [CrossRef]  

205. Chen, A.; Seneay, J.; Gödde, N.; Kinwel, T.; Ham, S.; Thompson, E.W.; Humbert, P.O.; Möller, A. Intermittent hypoxia induces a metastatic phenotype in breast cancer. Cancer Metastasis Rev. 2016, 35, 655–667. [CrossRef]  

206. Casazza, A.; Di Conza, G.; Wenes, M.; Finisguerra, V.; Deshoemaecker, S.; Mazzone, M. Tumor stroma: A complexity dictated by the hypoxic tumor microenvironment. Oncogene 2014, 33, 1743–1754. [CrossRef]  

207. Laitala, A.; Erler, J.T. Hypoxic Signalling in Tumour Stroma. Front. Oncol. 2018, 8, 189. [CrossRef]  

208. Aceto, N.; Bardia, A.; Miyamoto, D.T.; Donaldson, M.C.; Wittner, B.S.; Spencer, J.A.; Yu, M.; Pely, A.; Engstrom, A.; Zhu, H.; et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 2014, 158, 1110–1122. [CrossRef]  

209. Cheung, K.J.; Ewald, A.J. A collective route to metastasis: Seeding by tumor cell clusters. Science 2016, 352, 167–169. [CrossRef] [PubMed]  

210. Hong, Y.; Fang, F.; Zhang, Q. Circulating tumor cell clusters: What we know and what we expect (Review). Int. J. Oncol. 2016, 49, 2206–2216. [CrossRef] [PubMed]
207. Yin, T.; Getsios, S.; Caldelari, R.; Kowalczyk, A.P.; Müller, E.J.; Jones, J.C.R.; Green, K.J. Plakoglobin suppresses keratinocyte motility through both cell-cell adhesion-dependent and -independent mechanisms. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5420–5425. [CrossRef] [PubMed]

208. Kitamura, T.; Qian, B.-Z.; Pollard, J.W. Immune cell promotion of metastasis. *Nat. Rev. Immunol.* **2015**, *15*, 73–86. [CrossRef] [PubMed]

209. Paffenhöhl, S.V.; Salvagno, C.; Ho, Y.-J.; Limjoco, M.; Baslan, T.; Tian, S.; Kulick, A.; de Stanchina, E.; Wilkinson, J.E.; Barriga, F.M.; et al. Senescence induction dictates response to chemo- and immunotherapy in preclinical models of ovarian cancer. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2117754119. [CrossRef] [PubMed]

210. Hao, X.; Zhao, B.; Zhou, W.; Liu, H.; Fukumoto, T.; Gabrilovich, D.; Zhang, R. Sensitization of ovarian tumor to immune checkpoint blockade by boosting senescence-associated secretory phenotype. *Science* **2021**, *24*, 102016. [CrossRef]

211. Tasdemir, N.; Banito, A.; Roe, J.-S.; Alonso-Curbelo, D.; Camiolo, M.; Tschaharganeh, D.F.; Huang, C.-H.; Aksoy, O.; Bolden, J.E.; Chen, C.-C.; et al. BRD4 Connects Enhancer Remodeling to Senescence Immune Surveillance. *Cancer Discov.* **2016**, *6*, 612–629. [CrossRef]

212. Mavrogonatou, E.; Pratsinis, H.; Kletsas, D. The role of senescence in cancer development. *Semin. Cancer Biol.* **2020**, *62*, 182–191. [CrossRef]

213. Luo, X.; Fu, Y.; Loza, A.J.; Murali, B.; Leahy, K.M.; Ruhland, M.K.; Gang, M.; Su, X.; Zamani, A.; Shi, Y.; et al. Stromal-Initiated Transition of Umbilical Cord Derived Mesenchymal Stem Cells to Carcinoma-Associated Fibroblasts through TGF-β1/Smad Pathway. *Differentiation of Umbilical Cord Derived Mesenchymal Stem Cells to Carcinoma-Associated Fibroblasts through TGF-β1/Smad Pathway.* *Neoplasia* **2016**, *14*, 136. [CrossRef]

214. Ruhland, M.K.; Loza, A.J.; Capietto, A.-H.; Luo, X.; Knolhoff, B.L.; Flanagan, K.C.; Belt, B.A.; Alsach, E.; Leahy, K.; Luo, J.; et al. Stromal senescence establishes an immunosuppressive microenvironment that drives tumorigenesis. *Nat. Commun.* **2016**, *7*, 11762. [CrossRef]

215. Ohuchida, K.; Mizumoto, K.; Murakami, M.; Qian, L.-W.; Sato, N.; Nagai, E.; Matsumoto, K.; Nakamura, T.; Tanaka, M. Radiation to Stromal Fibroblasts Increases Invasiveness of Pancreatic Cancer Cells through Tumor-Stromal Interactions. *Cancer Res.* **2004**, *64*, 3215–3222. [CrossRef]

216. Birchmeier, C.; Birchmeier, W.; Gherardi, E.; Vande Woude, G.F. Metastasis, motility and more. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 915–925. [CrossRef]

217. Orr, F.W.; Wang, H.H. Tumor cell interactions with the microvasculature: A rate-limiting step in metastasis. *J. Cell. Biol.* **2001**, *151*, 173–182. [CrossRef] [PubMed]

218. Vande Woude, G.F. Tumor cell interactions with the microvasculature: A rate-limiting step in metastasis. *Cancer Res.* **2001**, *61*, 1189–1192. [CrossRef] [PubMed]

219. Chen, C.-C.; et al. BRD4 Connects Enhancer Remodeling to Senescence Immune Surveillance. *Cancer Res.* **2016**, *76*, 1725–1739. [CrossRef] [PubMed]

220. Ekström, E.J.; Bergenfelz, C.; von Bülow, V.; Serifler, F.; Carlemalm, E.; Jönsson, G.; Andersson, T.; Leandersson, K. WNT5A induces motility through both cell–cell adhesion-dependent and -independent mechanisms. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5420–5425. [CrossRef] [PubMed]

221. Yi, L.; et al. Senescence induction dictates response to chemo- and immunotherapy in preclinical models of ovarian cancer. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2117754119. [CrossRef] [PubMed]

222. Baalbaki, J.; et al. Senescence induction dictates response to chemo- and immunotherapy in preclinical models of ovarian cancer. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2117754119. [CrossRef] [PubMed]

223. Kim, Y.; et al. Senescence induction dictates response to chemo- and immunotherapy in preclinical models of ovarian cancer. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2117754119. [CrossRef] [PubMed]

224. Webber, J.; Steadman, R.; Mason, M.D.; Tabi, Z.; Clayton, A. Cancer Exosomes Trigger Fibroblast to Myofibroblast Differentiation. *Cancer Res.* **2010**, *70*, 9621–9630. [CrossRef] [PubMed]

225. Kim, J.; Kim, T.Y.; Lee, M.S.; Mun, J.Y.; Ihm, C.; Kim, S.A. Exosome cargo reflects TGF-β1-mediated epithelial-to-mesenchymal transition (EMT) status in A549 human lung adenocarcinoma cells. *Biochem. Biophys. Res. Commun.* **2016**, *468*, 643–648. [CrossRef]

226. Gu, J.; Qian, H.; Shen, L.; Zhang, X.; Zhu, W.; Huang, L.; Yan, Y.; Mao, F.; Zhao, C.; Shi, Y.; et al. Gastric Cancer Exosomes Trigger Differentiation of Umbilical Cord Derived Mesenchymal Stem Cells to Cancer-Associated Fibroblasts through TGF-β/Smad Pathway. *PLoS ONE* **2012**, *7*, e52465. [CrossRef]

227. Chen, X.; Liang, H.; Zhang, J.; Zen, K.; Zhang, C.-Y. Secreted microRNAs: A new form of intercellular communication. *Trends Cell Biol.* **2012**, *22*, 125–132. [CrossRef]

228. Zhao, Y.; Dong, Q.; Li, J.; Zhang, K.; Qin, J.; Zhao, J.; Sun, Q.; Wang, Z.; Wartmann, T.; Jauch, K.W.; et al. Targeting cancer stem cells and their niche: Perspectives for future therapeutic targets and strategies. *Semin. Cancer Biol.* **2018**, *53*, 139–155. [CrossRef]
259. Wei, L.-H.; Kuo, M.-L.; Chen, C.-A.; Chou, C.-H.; Lai, K.-B.; Lee, C.-N.; Hsieh, C.-Y. Interleukin-6 promotes cervical tumor growth by VEGF-dependent angiogenesis via a STAT3 pathway. *Oncogene* **2003**, 22, 1517–1527. [CrossRef]

260. Butti, R.; Nimma, R.; Kundu, G.; Bulbule, A.; Kumar, T.V.S.; Gunasekaran, V.P.; Tomar, D.; Kumar, D.; Mane, A.; Gill, S.S.; et al. Tumor-derived osteopontin drives the resident fibroblast to myofibroblast differentiation through Twist1 to promote breast cancer progression. *Oncogene* **2021**, 40, 2002–2017. [CrossRef]