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Whom to blame for metastasis, the epithelial–mesenchymal transition or the tumor microenvironment?

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Abbreviations: EMT, epithelial-to-mesenchymal transition; CSCs, cancer stem cells; TME, tumor microenvironment; TAMs, tumor-associated macrophages; CAFs, cancer-associated fibroblasts; m-car, mesenchymal carcinoma cell; e-car, epithelial carcinoma cell.

ABSTRACT

Changes in the tumor microenvironment (TME) can trigger the activation of otherwise non-malignant cells to become highly aggressive and motile. This is evident during initial tumor growth when the poor vascularization in tumors generates hypoxic regions that trigger the latent embryonic program, epithelial-to-mesenchymal transition (EMT), in epithelial carcinoma cells (e-cars) leading to highly motile mesenchymal-like carcinoma cells (m-cars), which also acquire cancer stem cell (CSC) properties. After that, specific bidirectional interactions take place between m-cars and the cellular components of TME at different stages of metastasis. These interactions include several vicious positive feedback loops in which m-cars trigger a phenotypic switch, causing normal stromal cells to become pro-tumorigenic, which then further promote the survival, motility, and proliferation of m-cars. Accordingly, there is not a single culprit accounting for metastasis. Instead both m-cars and the TME dynamically interact, evolve and promote metastasis. In this review, we discuss the current status of the known interactions between m-cars and the TME during different stages of metastasis and how these interactions promote the metastatic activity of highly malignant m-cars by promoting their invasive mesenchymal phenotype and CSC properties.

Introduction

Metastasis is a complex cascade of events that is the leading cause of cancer mortality. At the initiation of metastasis, cancer cells activate the latent embryonic program, called epithelial to mesenchymal transition (EMT), within the primary tumor, either as a result of underlying mutations or changes in the epigenetic landscape and the tumor microenvironment (TME) [1]. Following EMT,

epithelial cells lose their adherence junctions and epithelial polarity, and acquire a mesenchymal phenotype with invasive properties. In addition to morphological changes, a subset of carcinoma cells acquires stem cell properties (CSCs) during EMT, which promote long-term tumor propagation, drug resistance, and metastasis [2,3]. The role of the TME in metastasis was first addressed by Stephen Paget in 1889 in his famous seed-and-soil theory. He noticed that certain tumor cells (seeds) preferred to grow in specific tissue microenvironments in selected organs (soil) [4]. This approach prompted intense investigations of the role of the TME in the regulation of different aspects of tumor development. Pleiotropic interactions between various components of the TME and tumor cells have been identified, which have led to the development of TME-targeted therapies. Instead of targeting tumor cells directly, one targets the tumor-promoting function of the TME, such as vascularization of the tumor (anti-vascular therapy), or harnesses the immune system to attack tumor cells (the emerging field of immune checkpoint therapies) [5].

In this review, we discuss the current knowledge about the capability of TME to induce EMT in epithelial-appearing carcinoma cells (e-cars) and how the bi-directional interactions between carcinoma cells that have undergone EMT and appear mesenchymal (m-cars) and the different components of the TME promote metastasis. Since EMT and CSC properties have been linked together in many different tumors, in this review we use both terms, m-cars and CSCs. When referring to the EMT-generated mesenchymal-appearing CSCs, we refer to them as m-cars, and then we use CSCs for cancer cells isolated from tumor tissues, which possess stem cell properties. In particular, we focus on the role of three aspects of the TME in the regulation of metastasis via bi-directional crosstalk: (1) hypoxia/tumor vascularization, (2) inflammation, and (3) mesenchymal stromal cells and the ECM. In addition, we briefly discuss the role of metabolic coupling between stromal cells.

Influence of TME on carcinoma cells and the induction of EMT

Tumors are highly heterogeneous, consisting of pheno and genotypically different carcinoma cells and many different stromal cell types that participate in the formation of the TME and eventually form together a complex tissue-like structure. The most abundant non-tumorigenic cell types are cancer-associated fibroblasts (CAFs), which reside in the tumor stroma and participate actively in the regulation of tumor development [6]. In addition to CAFs, other mesenchymal stromal cells that reside in or are recruited to the TME, such as human mesenchymal stem cells [7] and adipocytes [8], have been shown to regulate many different aspects of tumor development.

Tumors also attract many cell components of the innate immune system, such as macrophages, myeloid-derived suppressor cells, monocytes, natural killer (NK) cells, and mast cells, as well as cell components of the adaptive immune system, such as killer T-cells, memory T-cells, and different types of B-cells. Proinflammatory activity is essential for tumor development, and chronic inflammation has been linked to tumor formation. On the other hand, the ability of tumor cells to dampen the immune system is essential for their survival and metastatic activity [9,10]. Another important cellular component of TMEs is endothelial cell, which participate in the vascularization of the tumor; this is essential for providing necessary nutrients and oxygen, and also for serving as a route for cancer cells to metastasize. During vascularization of the tumor, crosstalk between tumor cells and endothelial cells takes place, and the interaction leads to increased vascularization of the tumor and induction of EMT in carcinoma cells [11,12]. In addition to cellular components, the TME

consists of a meshwork of secreted proteins that modify the extracellular matrix (ECM) composition and the biomechanical properties of the underlying stromal tissue. Also oxygen status and pH are important aspects of TME [13,14]. The complex network of different interactions between invasive m-cars and various components of the TME occurs already during tumor progression and at every stage of the metastatic cascade. Therefore, both TME and m-cars contribute to metastasis in a synergistic fashion. The changes in oxygen levels leading to hypoxic regions, an early event in the tumor growth, can induce EMT and promote the generation of m-cars and thus enhance the vicious crosstalk between TME and m-cars leading to metastasis (Fig. 1).

Hypoxia and neovascularization in promoting EMT and metastasis

Oxygen status is an important component of the TME, and partial oxygen pressure varies considerably among different tumor sites [13–16]. The molecules that are responsive to low oxygen pressure are hypoxia-inducible factors (HIFs), which consist of oxygen sensitive HIF-α and oxygen-insensitive HIF-β [reviewed in 17,18]. Of the three distinct HIF-α proteins, HIF-1α is the most widely studied molecule regulating the hypoxic response [17–19]. Accordingly, the pathophysiological roles of HIF-1α and hypoxia have been well demonstrated in many solid tumors [20–22]. Interestingly, recent studies have revealed that HIF-1α, alone or in cooperation with other transcription factors, directly activates many EMT/CSC-related transcription factors such as TWIST [23], SNAI1 [24], Zeb-1 [25,26], BMI1 [27], and NOTCH [28], which are essential for hypoxia-induced invasion and metastasis. In addition to transcription factors, HIF-1α-mediated regulation of microRNAs or epigenetic modulators [29–31] promotes EMT. Accordingly, the local hypoxic microenvironment can activate the EMT program leading to the generation of m-cars, which further can promote the vascularization of the tumor (described below) and facilitate metastasis of these cells (Fig. 1).

M-cars promote neovascularization within the tumor

Interestingly, m-cars, which possess CSC properties, can promote tumor vascularization by transdifferentiating into endothelial cells, thus contributing in part to the tumor endothelium [32–37]. This process is well studied in glioblastoma, which is rich in CSCs [32–36, 38]. Ricci-Vitiani et al. [38] demonstrated that a major subset of tumor endothelial cells contains the same genomic alterations as tumor cells, indicating that they originate from the tumors. Moreover, xenograft experiments with human glioblastoma cells in immunocompromised mice showed that a major part of the endothelial cells was of human origin [38]. Bussolati et al. [39] isolated breast CSCs by serial passaging in mammospheres that were OCT-4 positive, but endothelial and epithelial markers negative. In the presence of vascular endothelial growth factor (VEGF), these cells formed vessel-like structures in vitro and expressed endothelial markers. More importantly, when cells from mammospheres were transplanted into immunocompromised mice, some of the endothelial cells inside the tumor were of human origin. Moreover, a well-known EMT inducer, TWIST1, is essential in head and neck carcinoma cell lines for inducing endothelial differentiation mediated via the Jagged-1-KLF4 axis [40]. Accordingly, m-cars with the CSC properties are capable of differentiating into endothelial cells and thus generating, at least, part of the tumor endothelium, although the functional significance of these cells remains to be determined.

Maniotis et al. [41] introduced a novel form of vascularization, in which cancer cells form a mesh-like ECM that resembles vessel-like tubes. This ECM enables blood flow and thus promotes neovascularization of the tumor [41–44]. This process, called vascular mimicry, has been observed

by many others, both in vitro and in vivo [42–56], primarily in aggressive and poorly differentiated tumors [43–46]. Vascular mimicry is also suggested to be clinically relevant as it is correlated with poor prognosis, at least in mesothelial sarcoma, breast cancer, and osteosarcoma [48,50,51]. Interestingly, many EMT-related transcription factors have been associated with regulation of vascular mimicry [52–55]. For example, hypoxia-induced expression of TWIST regulates vessel-like formation to form a mesh-like ECM that mimics the vasculature and promotes neovascularization inside the tumor [53]. In addition, Slug and Zeb2 regulate the vascular mimicry of cancer cells, indicating that EMT-generated m-cars play a role in this process [54,55].

M-cars are also capable of promoting vascularization within primary tumors by secreting proangiogenic factors, such as VEGF, interleukin (IL)-8, and fibroblast growth factor (FGF) [57–59]. VEGF is especially important for pro-angiogenesis, and the EMT-signaling pathways are driving its expression and secretion [57,58]. Moreover, SNAIL directly regulates the expression of IL-8, which was necessary for the SNAIL-driven CSC properties of colorectal cancer cells [56]. Moreover, Fantozzi et al. [58] demonstrated that VEGFA secretion by EMT-generated murine CSCs is critical to their tumor initiation capacity. Collectively, these findings suggest that following EMT-induction m-cars promote the tumor vascularization to promote metastasis as well as survival by enhancing the nutrient and oxygen supply. Furthermore, the tumor-associated vasculature is hyper-permeable compared to normal vessels enabling cancer cell extravasation and leakage of macromolecules. Therapeutic approaches aimed at “vascular normalization” to reduce microvascular permeability and inhibit tumor progression have been suggested as a potential anti-tumor strategy [59].

Bi-directional communication between m-cars and endothelial cells promotes permeabilization of the vessels

The bidirectional crosstalk between invasive m-cars and endothelial cells promotes the permeabilization of the endothelium and disrupts the cell–cell junctions between endothelial cells to promote intravasation and extravasation [60–63]. The secretion of VEGF itself can promote endothelial permeability, but several other mechanisms are also involved in this process [60]. The secretion of miRNA105 by metastatic cancer cells was recently shown to destroy cell–cell junctions between endothelial cells by targeting ZO-1 expression and allowing more efficient cancer cell metastasis [62]. Moreover, mechanisms that promote direct contact between cancer cells and endothelial cells have been reported, such as the α2β1 integrin complex-mediated interaction with VE-cadherin, which leads to phosphorylation of VE-cadherin on the surface of endothelial cells and the loss of cell–cell junctions [63]. These mechanisms, among others, promote the dissemination of m-cars from the primary site. After they leave the primary site, they also lose the signals from the primary tumor that promotes EMT and survival. Accordingly they need to find alternative mechanisms to maintain the m-car phenotype.

Crosstalk between endothelial cells and cancer cells promotes EMT/CSC-properties and survival of disseminating m-cars

Endothelial cell-mediated signaling, such as the secretion of epidermal growth factor (EGF) and Jagged, induces EMT/CSC properties in head and neck carcinoma and colorectal cancer cells [11,64]. This interaction promotes the self-renewal and survival of m-cars at the primary site. On the other hand, the interaction between disseminated tumor cells and endothelial cells following extravasation is necessary for colonization at the distant site. Endothelial cells residing in a stable

microvasculature secrete thrombospondin-1 promoting quiescence of disseminated tumor cells [65]. Conversely, sprouting endothelial cells of the neovasculature secrete periostin and transforming growth factor (TGF)-β1 promoting proliferation of disseminated tumor cells [65]. Moreover, Valiente et al. [66] reported that the L1 cell adhesion molecule (L1CAM)-mediated interaction of disseminated cancer cells and endothelial cells in brain capillaries facilitated the dispersal of disseminated tumor cells. In addition to expressing L1CAM, disseminated cancer cells secrete serpins that inhibit the plasmin-mediated activation of astrocytes and thus prevent the secretion of pro-apoptotic FasL. Taken together, the interplay between endothelial cells and m-cars is important not only for the acquisition and maintenance of EMT/CSC properties but also for colonization at distant sites.

Bidirectional interaction between carcinoma cells and immune cells within the TME

The role of inflammation in tumor development has been well studied for many decades. Tumor cells need the pro-inflammatory environment, which provides many signaling molecules that promote the m-car phenotype. However, partial dampening of the immune system is also necessary to avoid attacks by the adaptive immune system during metastasis [67]. Accordingly, invasive m-cars have developed several mechanisms for harnessing the immune system for their benefit (Fig. 2).

Inflammation induces EMT

Several pro-inflammatory factors promote EMT, CSC properties, and cancer invasiveness [68–73]. In addition to the EMT-inducing inflammatory cytokines, TGF-β1, tumor necrosis factor (TNF)-α and IL-6 have also been shown to enhance TGF-β1-mediated EMT [69,70]. Moreover, TNF-α- and IL-6-mediated activation of nuclear factor (NF)-κB is known to regulate many EMT transcription factors, such as ZEB1, SNAI1, and TWIST [71,72]. As one of the most important players in the innate immune system, tumor-associated macrophages (TAMs) regulate tumor aggressiveness and the EMT phenotype in many different cancer types, along with other members of the innate immune system, such as NK cells, mast cells, and monocytes [73–79]. The secretion of IL-8 by TAMs activates the JAK/STAT3/SNAIL signaling pathway in cancer cells and ultimately leads to the induction of EMT as well as the generation of m-cars [74]. Moreover, a feedforward loop exists between m-cars and TAMs. Tumor cells secrete granulocyte macrophage colony-stimulating factor (GM-CSF) to activate TAMs, which begin to secrete chemokine (C-C motif) ligand 18 (CCL 18), and in turn maintain the EMT phenotype of m-cars [76]. In addition to secreted soluble factors, several crosstalk mechanisms involving direct cell–cell contacts between cancer cells and TAMs have been observed. Lu et al. determined that CD90 and EphA4 on the surface of m-cars interact with the ligands that are expressed on the cell surface of TAMs and monocytes [78]. Activation of EphA4 further activates Src and NF-κB, which leads to increased expression of IL-6, IL-8, and GM-CSF. This generates a stem cell niche that helps maintain the CSC phenotype; in addition, secreted IL-6, IL-8, and GM-CSF promote the activation of TAMs, leading to a positive feedback loop [76,78]. Taken together, the bidirectional interactions between TAMs and m-cars result in a vicious cycle that maintains tumor aggressiveness and metastatic growth. These interactions between the innate immune system and m-cars help maintain the invasive phenotype, but m-cars have other mechanisms for evading the adaptive immune system and becoming invisible to different types of T-cells; these mechanisms help these cells survive during metastasis by avoiding attacks by T-cells.

Immune escape of m-cars

Immune checkpoints consist of molecules that either activate or dampen immune cell activity [reviewed in 80]. To become activated different types of T-cells of the adaptive immune system (CD8(+)-killer T-cells and CD4(+)-regulatory T-cells) need to interact with antigen-presenting cells [81]. In addition to the MHC class of molecules that presents antigens on antigen-presenting cells (APCs), T-cells require additional receptor–ligand-mediated interactions to become fully activated [80,81]. These include activating cell surface molecules B-7 and B-17 on the surface of APCs, which interact with the CD28 receptor expressed on T-cells [80]. In addition to antigens originating from pathogens, tumor cells generate a significant number of mutated proteins that the immune system considers "antigens outside the body"; many of these proteins also bind to the MHC class of molecules and are presented to T-cells [82,83]. The identification of inhibitory immune checkpoint molecules has led to more promising immune therapies [84,85]. The therapeutic antibodies against inhibitory checkpoint molecules expressed on T-cells, such as CTLA-4 and PD1, as well as therapeutic antibodies against the ligand of PD1, B-7–family member PD-L1, which is expressed in tumor cells [86,87], have shown very promising results in early clinical trials [88–95]. Interestingly, Alsuliman et al. recently showed that the expression of PD-L1 is tightly linked to EMT in claudin-low breast cancer cells [86]. Moreover, Chen et al. demonstrated that the expression of PD-L1 is regulated by the ZEB1-miRNA200 axis [87]. They found that mir200 represses the expression of PD-L1 in human lung cancer cells and that the well-known mir200 repressor, ZEB1, is important for upregulating PDL1 expression in EMT-induced cells. Moreover, PD-L1 expression was important to CD8(+)-mediated immunosuppression during metastasis. Targeting PD-L1 inhibited tumor growth and metastasis, indicating the importance of PD-L1-mediated immune escape of m-cars [87]. Taken together, PD-1/PD-L1-mediated crosstalk with T-cells protects m-cars from the adaptive immune system and helps them survive during metastasis.

Crosstalk between circulating tumor cells and platelets

For disseminating cells the transition from the primary tumor to the circulation is drastic, since primarily adherent cells need to survive in an anchorage-independent environment where they also encounter many harsh physical stresses. Not surprisingly, circulating tumor cells (CTCs) also use the vasculature TME to maintain an invasive EMT phenotype and colonize distant hostile sites [96,97]. The interaction between platelets and CTCs is important for at least two reasons. First, it promotes the metastatic activity of CTCs, and secondly the crosstalk promotes survival of disseminated cancer cells. Direct cell–cell interaction with CTCs and platelets induces secretion of TGF-β1 in platelets which activate both the TGF-β/SMAD and NF-κB signaling pathways in CTCs and helps maintain their EMT phenotype and metastatic activity [96]. Another example of platelets facilitating metastasis is that upon contact with disseminated tumor cells, platelets secrete chemokine (C-X-C motif) ligand 5 (CXCL5) and chemokine (C-X-C motif) ligand 7 (CXCL7), which further recruit granulocytes to the early metastatic niche promoting the survival of disseminated tumor cells and colonization of the metastasis site [97]. The depletion of platelets and granulocytes and blocking the CXCL5/7 receptor CXCR2 on the cell surface of granulocytes are capable of inhibiting the metastatic outgrowth of cancer cells [97].

The role of TME in regulation of reverse EMT process called mesenchymal to epithelial transition (MET)

In some cancer types the metastatic lesions remarkably resemble the differentiated tissue of origin. For example, breast cancer metastases appear as islands of epithelial-like breast tissue in the setting of a distant organ like liver [98,99]. Such findings have brought forward the notion that following extravasation disseminating m-cars would undergo a reversal of EMT, namely mesenchymal to epithelial transition (MET). In contrast to EMT, which is fairly well understood at the molecular level, at least in vitro, the process of MET remains poorly understood. Gene expression profiling has indicated that human primary breast tumors are strikingly similar to the distant metastases of the same patient [100]. However, recent DNA sequencing has suggested that metastatic cells can also harbor unique mutations not detected in the primary tumors [101]. The potential role of MET was demonstrated recently in a paper focusing on identification of the phenotype of metastatic cells in mouse xenograft model [102]. Single-cell sequencing of metastatic cells shows that low-burden metastatic tumor includes cells with distinct stem cell and EMT-associated gene expression properties, whereas high-burden metastatic tumor contains cancer cells with similar gene expression signature with that of the primary tumor [102]. This supports the important role of EMT-generated CSCs during early metastasis and the role of MET during the formation of macrometastases. Given the strong evidence for EMT in breast cancer progression, this suggests that different TME environments may dictate the tumor cell phenotype such that transition between EMT and MET is facilitated by the TME of the metastatic niche. One such example is the ability of bone marrow-derived extracellular matrix component chondroitin sulfate proteoglycan versican to promot lung metastasis of breast cancer cells by inducing MET in the disseminated cells [103]. Moreover, the re-expression of E-cadherin has been shown in E-cadherin-negative primary tumors that have metastasized into liver [104]. Interestingly, E-cadherin expression was highest in metastasized tumor cells that reside next to hepatocytes [104]. This interaction was further studied in vitro in co-culture experiments and indeed the interactions between the breast cancer cell line MDA-MB-231 and hepatocytes induced re-expression of E-cadherin in MDA-MB-231 cells, suggesting the potential role of hepatocytes in regulation of MET and metastasis outgrowth [104]. While all of this evidence supports the notion about the role of TME during the MET, there is still a need for a detailed investigation to be firm.

Role of the mesenchymal stromal cells and ECM components in the induction of EMT and metastasis

The ECM consists of highly abundant secreted fibrillary proteins and bioactive molecules that form the biophysical support and also provides necessary cues for tumor growth. Both the tumor cells and stromal cells participate in the formation of the ECM, and the crosstalk between these cells modifies the biomechanical properties of the ECM. In fact, the tumor-suppressive ECM found in normal tissues becomes tumor supportive during tumor progression. In addition to modifying the ECM, mesenchymal stromal cells can also activate the EMT-program in cancer cells via different mechanisms, which involve paracrine mechanisms, direct cell–cell contacts and also secreted exosomes (Fig. 3).

The bidirectional crosstalk between CAFs/hMSCs and cancer cells forms a vicious cycle leading to metastasis

The conversion of normal fibroblasts to CAFs by TGF-β has been shown to play a significant role in tumor progression [105–110]. In fact, an autocrine loop of TGF-β1 and stromal cell derived factor-1 (SDF-1) was demonstrated in CAFs during breast cancer development [109]. Disruption of this autocrine loop attenuated myofibroblast-like CAF morphological features and the tumor-promoting function of surrounding fibroblasts. In addition, in colorectal cancer TGF-β induced generation of CAFs promotes metastasis of the cancer cells [110]. In addition to TGF-β1, CAFs secrete other EMT-inducing factors such as tumor necrosis factor-alpha (TNFα) [111], fibroblast growth factor (FGF) [111], chemokine (C-X-C motif) ligand 12 (CXCL12) [111], chemokine (C-C motif) ligand 12 (CCL2) [112], and matrix metalloproteinases (MMPs) [111]. On the other hand, m-car-mediated secretion of FGF, TGF-α, and CXCL1-4 converts normal fibroblasts into CAFs, generating a vicious cycle between m-cars and CAFs that maintains tumor-supportive activity [111]. These findings highlight the complexity of tumor–TME interactions, and defining the initiating event. Is it m-car mediated activation of normal fibroblasts into CAFs leading to the generation of tumor promoting ECM, or the phenotypic change from normal fibroblasts to CAFs that in turn generate EMT-promoting ECM leading to the formation of m-cars? Most likely both scenarios are occurring in parallel, although there is little evidence for genetic changes in the stroma as the initiating event.

While the role of CAFs in tumor development is widely known, the influence of human mesenchymal stem cells (hMSCs) is more complex and controversial [113–118]. hMSCs are recruited from the circulation to tumors by TGF-β1, secreted by CSCs [119]. However, whether they promote [113–116] or inhibit [118] tumor development depends on the type of hMSCs and other cues coming from the TME, especially from the immune system [116–118]. hMSCs are active modulators of the immune system and are very responsive to many pro-inflammatory molecules. For example, TNF-α-primed hMSCs inhibit tumor development via DDK3 and TRAIL that induces growth arrest and apoptosis in tumor cells [118]. In contrast, others have shown that immunosuppression mediated by hMSCs promoted tumor growth and development [116]. Moreover, the secretion of hepatocyte growth factor and SDF-1 by hMSCs promotes tumor growth and aggressiveness [113–115]. More detailed studies are needed to better identify the tumor-promoting and -inhibiting roles of hMSCs given that they are widely used in cellular therapies for several autoimmune and degenerative diseases.

Altered ECM stiffness induces EMT in epithelial carcinoma cells and helps maintain invasive m-car phenotype

In addition to direct crosstalk between CAFs and tumor cells, the transformation of normal fibroblasts to CAFs also leads to significant changes in secreted ECM proteins, promoting the formation of a disorganized, denser, and stiffer ECM [105]. It is known that cancer cells can respond to alterations in the ECM, leading to changes in their gene expression, proliferation and EMT phenotype [120–122]. For example, the rigidity of the matrix regulates the TGF-β responsiveness of cells [120]. A more rigid ECM correlates with greater induction of EMT after TGF-β treatment, whereas a less rigid ECM results in TGF-β-mediated apoptosis. This finding highlights the importance of the ECM as a determinant for context-dependent cellular responses. Moreover, other studies have shown that modification of the biophysical properties of synthetic matrixes can regulate the EMT of cancer cells [121]. Moreover, it was demonstrated that the ECM secreted by normal

fibroblasts, SNAI1-positive CAFs and SNAI1-negative CAFs, led to differential changes in the stiffness and architecture of the ECM [105]. The SNAI1-positive CAFs promoted the invasive properties of tumor cells by modulating the ECM properties, making them more invasive than the ECM secreted by normal fibroblasts or SNAI1-negative CAFs. Most recently, Wei et al. demonstrated that nuclear localization of TWIST was supported by ECM stiffness, linking TWIST-mediated transcriptional regulation to mechanosensing and providing a mechanism for stiffness induced EMT [122]. Even though tumor hypoxia has been associated with increased metastasis, more aggressive tumors as well as EMT (as discussed previously in this review), recent studies have reported an opposite role of hypoxia. Interestingly, chronic hypoxia was shown to hinder the tumor-promoting function of CAFs [123]. Chronic hypoxia led to suppressed matrix remodeling by CAFs and eventually to decreased tumor stiffness and metastasis. This highlights the role of ECM in the regulation of metastasis, and also illustrates the complexity of the role of hypoxia-mediated changes in TME that eventually results in tumor development.

Exosome-mediated crosstalk between m-cars and TME

Interestingly, several recent reports in pancreatic cancer, melanoma, and renal cancer have shown that exosomes secreted by tumor cells can modify the microenvironment at distant sites of metastasis, making it more susceptible to colonization [124–127]. Tumor cell-secreted exosomes enter the circulation and home to distant sites, where they can modify the ECM of the host microenvironment by degrading the ECM; promote the secretion of specific ECM proteins [124], such as fibronectin [125]; or promote angiogenesis [126]. Until recently, the mechanism of exosome-mediated generation of specific metastatic niches was not well understood. However, identification of a family of cell adhesion receptors, integrins, enriched on metastasis-promoting exosomes, suggested that integrin-mediated tethering of tumor-derived exosomes might enable their homing and enrichment to certain favorable matrix environments to generate metastatic niches at distant sites [128]. In addition, short distance exosome-mediated crosstalk between mesenchymal stromal cells and m-cars has been shown to promote dormancy, drug resistance, and m-car migration and metastasis [129–131]. On the other hand, cancer cell-derived exosomes enhanced the transition from fibroblasts to pro-tumorigenic myofibroblasts [132–134]. To conclude, metastatic tumor cells are capable of modifying the metastatic niche to become more susceptible to colonization even before they reach the distant site.

Metabolic coupling between m-cars and stromal cells

The Warburg effect, also known as aerobic glycolysis, is a well-known phenomenon in cancer, even though more detailed studies about the interactions between stromal and cancer cells have cast doubt on its effect, and a new hypothesis, the reverse Warburg effect, has been postulated [135,136]. During the reverse Warburg effect, tumor cells induce metabolic stress in neighboring stromal cells leading to aerobic glycolysis and increased autophagy in stromal cells. As a consequence, stromal cells begin to secrete high energy-rich metabolites, such as lactate and pyruvate, into the TME for utilization by tumor cells. This also leads to more aerobic metabolism in tumor cells to promote their proliferation, which is opposed to Warburg's observation. Nevertheless, aerobic glycolysis seems to be common in EMT-generated cells and highly aggressive TNBCs, which are enriched for m-cars [137–142]. Upon EMT, carcinoma cells reprogram their metabolisms and become more dependent on glycolysis [143–145]. The reason for the switch in

metabolism is not well understood; however, aerobic glycolysis seems to be common in normal tissue-specific stem cells as well [146–148], suggesting that it is a common and important feature of stem cells in general. This metabolic plasticity may also offer a growth advantage under highly variable growth conditions, from poorly oxygenized regions to highly nutrient-rich environments, and provide an advantage during anti-vascular therapies [149–151]. This highlights the importance of the metabolic adaptation of tumor cells in response to changes in the TME. The metabolic coupling of cancer cells with stromal cells has mostly been studied in epithelial cancers. Recent studies have indeed shown that the reverse Warburg effect may be more prevalent in Luminal-A breast cancer, whereas TNBCs possess a more typical Warburg effect or mixed phenotype, however, more detailed studies are needed to confirm the role of reverse Warburg effect in breast cancer [152–155]. Regardless, EMT-generated CSCs can also use many products of glycolytic pathways from the extracellular space, which demonstrates that EMT-generated CSCs use the metabolites from the adjacent stroma [156]. In fact, the metabolic coupling between metastatic ovarian cancer cells and adipocytes at the omentum promotes the survival of disseminated tumor cells at the distant site and colonization [157]. In addition, metastatic prostate cancer cells have been shown to use the lipids secreted by adjacent adipocytes [158]. These interesting observations clearly demonstrate that the metabolic coupling between tumor cells and adjacent stromal cells is an important aspect of the tumor–TME crosstalk that promotes tumor development.

Conclusions and future directions

Our understanding of the role of TME in the regulation of different aspects of tumor initiation and progression has increased tremendously. The bidirectional crosstalk between the TME and m-cars occurs in every stage of the metastatic cascade, but which initiates the vicious cycle is extremely hard to pinpoint because both the TME and m-cars are culprits for the metastasis. The role of hypoxia in the induction of EMT in solid tumors is well characterized and could be the initial impetus for the generation of m-cars, which can then further modify the TME to become even more permissive for metastasis. Regardless, a better understanding of the role of TME in the regulation of tumor development has led to the many promising TME-targeting therapies, the latest example of which is immune checkpoint therapy. Despite this progress, the detailed molecular mechanisms behind the bidirectional crosstalk between TME and m-cars are still mainly unknown, limiting the potential of TME-targeting therapies. Since the TME evolves together with the tumor and is always changing, a more detailed characterization of the interactions and the molecular mechanisms of m-car TME crosstalk at different stages of cancer development should help shed light on the influence of EMT on TME and vice versa.

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Conflict of interest

The authors declare no conflict of interests.

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Figures and Figure Legends

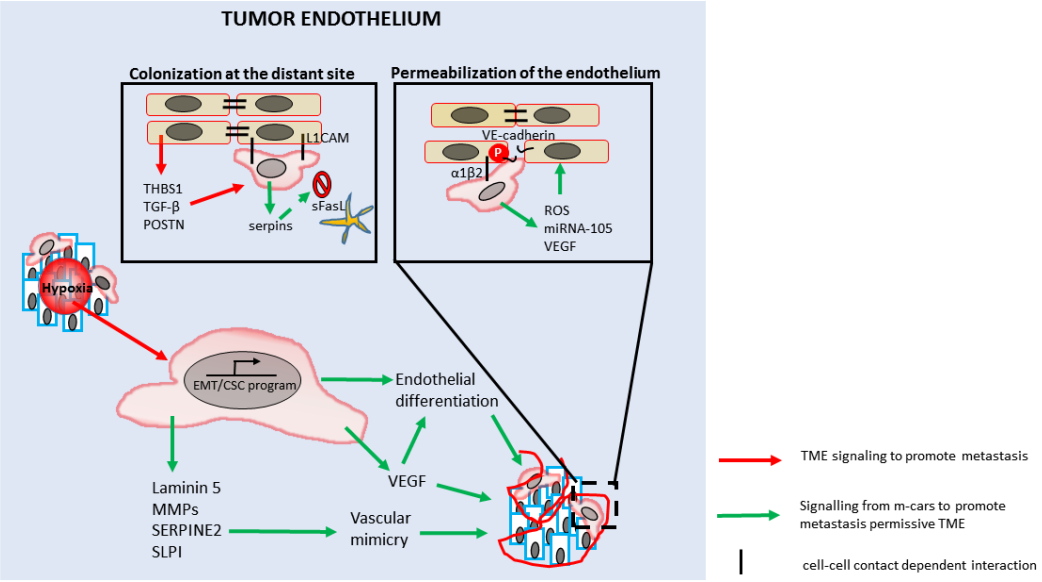

Fig. 1. Crosstalk between tumor endothelium and tumor cells after hypoxia-induced EMT. Hypoxia is known to induce EMT in epithelial carcinoma cells (e-cars) mostly via HIF-1α. This leads to upregulation of EMT-related transcription factors, such as Twist, Zeb1, Snail, Slug, and BMI1. Upon EMT-induction, epithelial carcinoma cells transform into mesenchymal carcinoma cells (m-cars) and acquire cancer stem cell (CSC) properties. M-cars have several mechanisms to induce tumor vascularization to promote their metastasis, and also to secure the supply of oxygen and nutrients to enable tumor growth. The crosstalk between endothelial cells and m-cars takes place in many steps of metastasis, such as during intravasation, extravasation and colonization at the distant site.

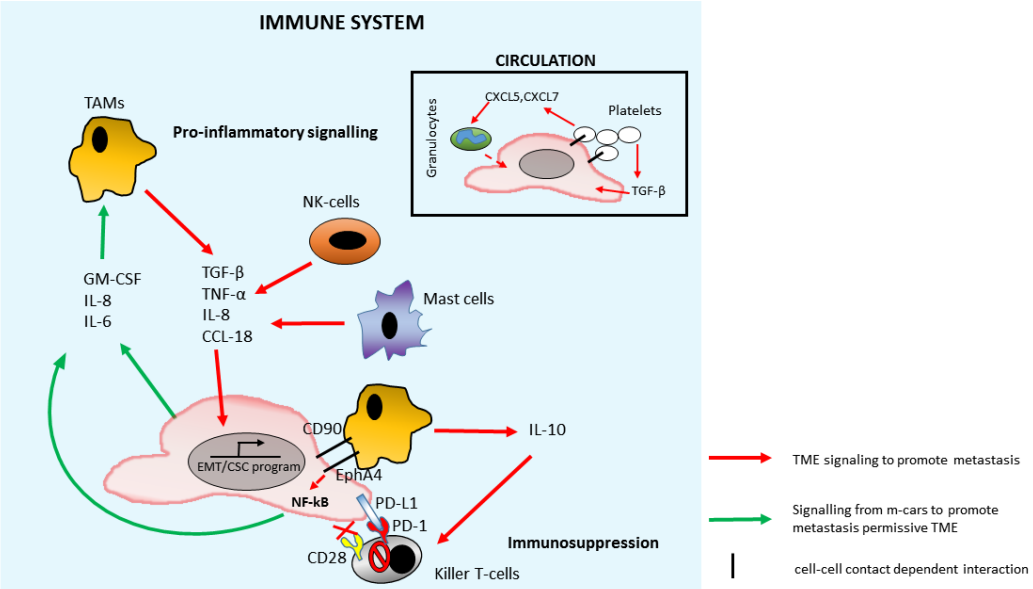

Fig. 2. Crosstalk between m-cars and the immune system. The cells from the innate immune system provide many EMT-inducing pro-inflammatory cytokines, which support the m-cars and can induce EMT in e-cars. Several vicious positive feedforward loops exist between m-cars and immune cells, such as the ability of tumor-associated macrophages to promote the metastasis of m-cars. Moreover, m-car–platelet interactions in circulation support the m-car phenotype and survival during the extravasation and colonization at the distant site. The balance between pro-inflammatory action and immunosuppression is necessary for metastatic cells. M-cars are also able to prevent the activation of killer T-cells and thus evade attacks of T-cells.

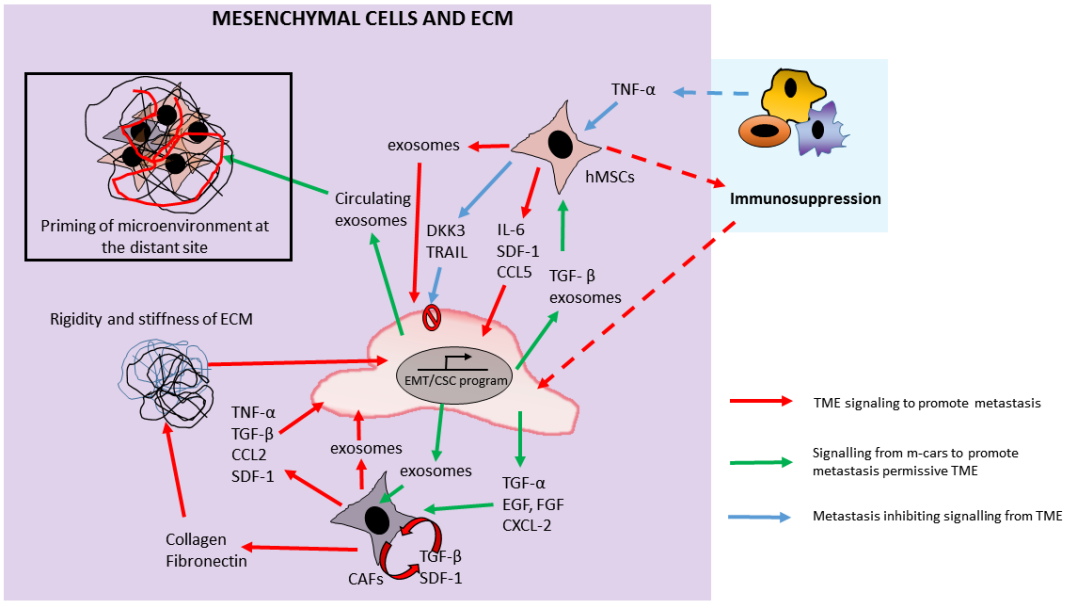

Fig. 3. Crosstalk between mesenchymal stromal cells and tumor cells promotes EMT and metastasis-permissive ECM. The most common cellular component of the TME is the cancer-associated fibroblast (CAF) that can promote the metastatic activity of m-cars and induce EMT in e-cars. The bidirectional crosstalk between tumor cells and CAFs leads to activation of normal fibroblasts into tumor promoting CAFs. Conversely CAFs can induce EMT and help m-cars to maintain their invasive phenotype via a paracrine function or by modifying the ECM to become more permissive for metastasis. In contrast to predominantly tumor promoting CAFs, human mesenchymal stem cells (hMSCs) can either stimulate (red lines) or inhibit (blue lines) the invasive m-car phenotype. Interestingly, m-cars are able to secrete exosomes that home to specific distant pre-metastatic sites, possibly via their surface exposed integrin receptors, and modify the microenvironment to become less hostile to metastatic cells. Also short-distance crosstalk between m-cars and mesenchymal stromal cells regulates the invasive phenotype of m-cars.