Mesenchymal Stem Cells During Tumor Formation and Dissemination

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Abstract The tumor microenvironment (TME) is composed by malignant and non-malignant cells, all embedded in a dense extracellular matrix (ECM) rich with unstable vessels. Targeting TME components, especially those associated with the vasculature such as endothelial cells (ECs) and pericytes, has shown clinical benefits. The identity correlation between pericytes and mesenchymal stem cells (MSC) has broadened the functional roles of these adult stem cells, now tightly involved in cancer biology. This review summarizes this involvement, focusing on their participation in: 1) skeletal primary malignancies; 2) formation of distant primary tumors; 3) intravasation of cancer cells at the primary tumors; and 4) extravasation of cancer cells at the target organ. Given their tropism to sites of injury and inflammation, bone marrow (BM)-derived MSC (BM-MS) follow tumor-derived signals and participate in the formation of distant primary tumors, by repopulating their perivascular habitat and contributing to tumor growth. Thus, targeting primary tumor's pericytes severely reduces growth, yet dissemination of constitutive cancer cells increases. The impact of pericyte-deficient coverage on the target organ is rather opposite, generating a selective reduction of cancer cell invasion in some organs. These roles seem to be founded on the distinct molecular communication

and physical interactions between MSC as pericytes and the cancer cells.

Keywords Mesenchymal Stem Cells (MSC) · Pericytes · Tumor microenvironment · Cancer dissemination · Intravasation · Extravasation

Introduction

Cancer (primary and metastatic tumors) constitutes the second cause of mortality after heart disease, according to the Centers for Disease Control and Prevention (CDC) (about 22 % in women and 24 % in men) [1]. Metastasis is considered a leading prognostic indicator for patient survival and a major contributor to the mortality associated with cancer, with the skeleton as one of the preferred organs for cancer metastatic dissemination in various primary tumors. Tumors are composed of a tight interplay between malignant and non-transformed cells embedded in a highly dynamic extracellular matrix (ECM) rich in unstable blood vessels, collectively constituting the tumor microenvironment (TME) [2]. The cellular compartment within the TME includes cells from the immune system, ECs, pericytes, adipocytes, MSC and tumor-associated fibroblasts (TAF), some of them arising from the tissue itself, while others are recruited from distant locations, mainly the bone marrow (BM) [3–6].

Current cancer therapies are designed to target not only specific biological functions in cancer cells, but also components of the tumor microenvironment (TME), especially the vasculature (i.e., ECs and pericytes), given its critical role in primary tumor growth and dissemination [7]. Pericytes are recruited close to the endothelium by ECs via PDGF-B signaling, where they attach and help stabilize the blood vessel [8, 9]. Pericytes constitute a novel therapeutic target based on

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recent evidence showing superior reduction in tumor growth when targeted in parallel with ECs [10]. However, pericyte coverage of the vasculature seems to have opposite effects on growth and metastatic potential of primary tumors [11, 12]. This has led to the appreciation of pericytes as gatekeepers for cancer metastasis [13]. With the increasing evidence of MSC residing in the perivascular space (as pericytes), new concepts regarding MSC involvement in the field of cancer are emerging. These concepts are provided in this review, divided in four main topics and with an emphasis on BM-MSC and on skeletal malignancies: 1) MSC in the BM as the cell of origin in various primary bone neoplasms; 2) The participation of BM-MSC during the formation of distant solid tumors; 3) The role of pericytes at the primary tumor's vasculature during intravasation of cancer cells; and 4) The regulatory role of MSC (as pericytes) at the target organ during cancer cell extravasation.

1) MSC as pericytes

Pericytes are typically defined as heavily branched cells embedded within the basement membrane of non-muscular microvessels, capillaries and postcapillary venules, and in close contact with the underlying endothelial cells. Initially identified by Rouget in 1863 as adventitial cells, pericytes have received a number of names, including mural cells, perivascular cells, periendothelial cells and deep cells [14]. During development-related vasculogenesis pericytes originate from MSC and neural crests, depending on the specific location [15]. In post-developmental stages, the origin of pericytes is far less clear, with tissue resident stem cells [16] and/or myofibroblasts [17], as well as peripheral pluripotent stem cells derived from the BM [16, 18] as potential progenitors. Pericytes have a broad spectrum of functions, including the regulation of the vessel's stability, permeability and tone, as well as the shift between quiescent and angiogenic stages of blood vessels, thus participating in the maintenance of the tissue homeostasis. In addition, pericytes participate in immunological defense reactions (e.g., macrophage-like properties), and help in the synthesis of the basement membrane [14, 19].

The description of the physical location of MSC *in vivo* has captured the attention of researchers for a long time. Initial studies in the early 1990s demonstrated that pericytes constitute the cell source for different mesenchymal tissues. For instance, Diaz-Flores et al. described venule pericytes in grafted perichondrium and periosteum as the drivers for neochondrogenesis in muscle [20] and as the source of osteoblasts in periosteal osteogenesis [21], respectively. In parallel, Brighton and colleagues proposed the pericyte as the possible progenitor of osteoblasts, based on *in vitro* studies in which phenotypic similarities, including matrix deposition and osteogenic markers, were established between them [22, 23]. This direct functional relationship between mesenchymal

progenitors (i.e., MSC) and pericytes was then confirmed with the detailed description of the mesenchymal stem cell niche, present in almost all post-natal organs and tissues, and in which both cell types share identity and functions [24–32]. Nevertheless, the cellular, molecular and physical complexity of the stem cell niche, along with descriptions of restricted differentiation by both MSC and pericytes, have suggested that the niche is populated by heterogeneous cell types and/or that not all pericytes are MSC [33].

Finally, this phenotypic/functional relationship between MSC and pericytes has also been evident with malignancies derived from the perivascular niche. Given their established mesenchymal potential, they have been associated with multiple soft tissues and bone-specific malignancies of mesenchymal origin [14], as well as with vascular primary tumor, such as the myopericytoma that exhibits a myoid differentiation pattern [34].

2) MSC in primary bone tumors

The most prevalent primary tumors in bone are Osteosarcoma (OS), Chondrosarcoma (CS) and Ewing's sarcoma (ES), all accounting for about 75 % of the cases [35•]. The cellular origin for these malignancies has been subject of intense investigation. In general, the resulting tumor phenotype is dictated by a combination of various factors, namely: the type of mutations accrued, the differentiation stage of the cell acquiring those mutations and the influence of the surrounding microenvironment [35•, 36]. For instance, specific mutations in undifferentiated MSC are responsible for the generation of ES. In contrast, mutations acquired in more specific subpopulations at different stages of differentiation (e.g., committed pre-osteoblasts) have been associated with the initiation of OS [35•].

Ewing's sarcoma is a primary bone and soft tissue tumor of mesenchymal origin. It is the second most frequent bone tumor, present almost exclusively in children and young adults. Its prognosis is directly correlated with its stage of detection, with a 5-year survival of 60–70 % [37]. The molecular pathogenesis of ES is defined by a recurrent chromosomal translocation between the EWSR1 gene and one of the five ETS transcription factors, which leads to the formation of a chimeric fusion gene [38, 39]. At least 80 % of the fusion is presented as the EWS/FLI1 oncoprotein. Mechanistically, this resulting aberrant transcription factor appears to promote abnormal cellular growth by transcriptionally modulating a complex network of target genes during differentiation, including *m-FNG*, PDGF-C ligand and TGFβRII [40], as well as disturbing RNA splicing and the ability of specific proteins to form functional complexes [41]. Historically, ES was thought to have originated from the endothelium (original proposition by James Ewing in 1921 and revisited in [42]). Later, a neuroectoderm origin was proposed, given that its

constitutive cells express neural markers and are capable of neural differentiation [43]. Recently, a more mechanistic theory has been suggested, in which not only a mesenchymal origin proposed, but also the disrupting signaling capacity of the aberrant transcriptional factor/oncoprotein in MSC is related to tumor development [44]. In that sense, EWS/FLI1 is capable of inhibiting differentiation of mesenchymal precursors, such as adipogenic and osteogenic from murine MSC [45], and myogenic from C2C12 myoblasts [46]. These *in vitro* results were complemented with reports of an *in vivo* transformation of BM-derived MSC capable of generating tumors in immunocompromised mice with features similar to ES. These features include a small cell size, expression of ES-identifying markers, dependence on insulin-like growth factor 1 (IGF-1), and induction or repression of various EWS/FLI1 target genes [38]. In parallel, the expression of ES markers such as CD99 has been detected in MSC derived from ES patients [47]. Altogether, these data point toward the MSC as the permissive cell of origin for ES, which after transformation by the aberrant oncogene gives rise to this poorly differentiated mesenchymal tumor. Interestingly, this mesenchymal theory can be reconciled with the vascular origin of ES cells as initially proposed by Ewing. First, it has been shown that EWS/FLI1 modulate a number of angiogenic genes; second, the native FLI1 gene is pivotal during the molecular regulation of blood vessel formation; and third, a clear vascular mimicry has been identified *in vivo* within ES tumors, where their derived cells show the capacity of forming vascular-like structures [48]. In conclusion, ES cells may derive from MSC, which in turn exhibit mesenchymal and vascular potentials.

Additional information supporting the role of MSC as initiators of ES comes from their direct relationship with cancer stem cells (CSC) within the tumor. Suvà et al. isolated a subpopulation of tumor cells from ES biopsies, based on the conserved CSC marker CD133. The resulting CD133^{Pos} population retained the MSC plasticity and showed the ability to initiate and maintain tumor growth through serial transplantation in immunodeficiency mice, in sharp contrast with their CD133^{Neg} counterparts. In addition to re-establish the parental tumor phenotype, the percentage of CD133^{Pos} cells in all subsequent tumors remained unaltered when compared with the original tumor, showing self-renewal capacity [49]. Collectively, these observations were consistent with CSC behavior. In another study, Riggi et al. demonstrated that the fusion gene/aberrant transcription factor EWS/FLI1 is responsible for “reprogramming” MSC into a phenotype compatible with ES CSC [50]. They reported the expression of the embryonic stem cell genes Sox2, Oct4 and NANOG in MSC derived from pediatric samples, a phenomenon not evidenced in their adult counterparts. Mechanistically, EWS/FLI1 represses the promoter

activity of miRNA145 on both itself and its in turn repressive activity over Sox2, demonstrating the effect of a single oncogene on reprogramming MSC into CSC.

Osteosarcoma is the most frequent tumor in bone, prevalent in adolescent and the elderly, which can be presented in a number of pathological variants depending on the stage of commitment of the osteoblastic cell [51]. The role of MSC as initiators of OS is less evident than with ES. In fact, more committed osteoprogenitors rather than undifferentiated MSC constitute the cell origin of OS. For instance, Rubio et al. reported that the excision of p53/Rb in BM-MSC generated leiomyosarcomas, while a similar deletion in further differentiated osteogenic cells generated OS-like tumors. Furthermore, they showed that the osteogenic differentiation stage of BM-derived MSC, not of cells derived from adipose tissue irrespective of their osteogenic stage, imposes the phenotype of *in vivo* OS development [52]. On the other hand, Shimizu et al. showed that BM-MSC from Ink4a/Arf KO mice displayed at least two different subpopulations with different OS formation capacities: a highly tumorigenic population with bi-potent (osteochondral) differentiation potential, and a low tumorigenic population with tri-potent (not restricted) potential. Interestingly, when adipogenic differentiation was enforced in the two populations, the OS formation potential was significantly impaired. The authors concluded that the chondrogenic and adipogenic intrinsic programs may suppress the OS potential of MSC [53]. Collectively, and unlike in ES where undifferentiated MSC act as the cell of origin, more committed osteoprogenitors constitute the cell source of OS. Furthermore, the closer to an undifferentiated MSC phenotype, the less likely an OS will be generated [54].

In addition to the aforementioned link of MSC (and their direct cellular descendants) with primary neoplasms in the bone, their key perivascular residence and vascular support functions enable them to also participate in distant tumor formation.

3) Bone marrow-derived MSC in distant primary tumor formation

Neovascularization is required for tumor growth [55]. The TME exhibits features of a site with chronic inflammatory conditions. This local inflammation, along with intratumoral hypoxia and the activity of recruited cells such as the tumor-associated macrophages (TAMs) and a myriad of pro-angiogenic molecules, drives the formation of new blood vessels [2, 56, 57]. However, constant formation, regression and remodeling of vascular structures dominate inside the tumor, generating unstable, leaky, tortuous, dilated and immature vessels [2, 14, 55]. The underlying imbalance between anti- and pro-angiogenic factors is accompanied by pericyte coverage deficiencies, which in conjunction contribute to the typical vascular abnormalities seen in tumoral vasculature [13,

58]. These deficiencies in pericytes include variable recruitment and coverage of the vasculature, dissociated pericyte-ECs physical interactions, and the secretion of an altered basement membrane [14, 59].

A number of reports have established that in response to tumor-derived molecular signals including inflammatory and hypoxic mediators, BM-derived progenitor cells of mesenchymal origin (BM-MSC) are recruited to the tumor stroma as tumor-associated fibroblasts (TAFs) [4–6, 16]. TAFs exhibit a myofibroblast phenotype and localize in both perivascular and parenchymal sites, contributing to the fibrovascular network expansion and tumor progression [60–62, 63•, 64•]. In addition, they have a particular secretory capacity associated with tumor formation, growth and distant dissemination potential, thus contributing to the ultimate clinical outcome [65, 66]. In that regard, they secrete factors associated with promoting cell growth, ECM remodeling, angiogenesis and tumor aggression, making them distinguishable from regular tissue resident fibroblasts [67]. Within the tumor fibrovascular network, two distinct populations of TAFs have been identified, recruited sequentially and based on the steps of tumor development. On the one hand, a pericytic population recruited from neighboring adipose tissue was involved in the constant neovascularization of the growing tumor. On the other hand, a fibroblastic population recruited from the distant BM related with ECM remodeling and tumor growth directly [68•].

In addition to MSC, the BM provides other cell sources that travel far to the tumor stroma, recruited by specific queues. For instance, VEGF-responsive BM-derived endothelial and hematopoietic precursors are necessary and sufficient to guarantee tumor angiogenesis [3, 69]. On the other hand, mobilization and recruitment of BM-derived PDGFR β +pericyte progenitor cells have also been described in tumors, presumably recruited under the influence of PDGF- β ligand [18, 70]. In conclusion, BM-derived cells are critical for tumor formation and growth, and subsequent invasive and metastatic potential.

4) Pericytes in cancer dissemination

Growth rate, invasion and metastasis in solid tumors constitute independent pathological traits regulated by different genetic mechanisms [71, 72]. Nevertheless, they share a common feature, the dependence on the TME and specifically the vasculature, as it provides access to oxygen and nutrients and serves as a dissemination route for malignant cells [7]. The metastatic process requires cancer cells to experience a series of steps, all potentially compromising their survival. These steps include escaping from the solid tumor to the vasculature (i.e., intravasation), traveling through the circulation and

finding a suitable soil to disseminate and continue growing after migrating out from the local blood vessels (i.e., extravasation) [73].

Targeting both EC and pericytes has proven to be more effective at reducing tumor growth than EC alone [10]. However, the microvascular structure, and specifically vessel coating pericytes, seems to differentially affect primary tumor growth and dissemination potential [13]. In fact, poor pericyte coverage of tumor vessels has been correlated with poor patient prognosis [74], due to increased metastatic dissemination, as shown in colorectal and prostate cancer [75, 76]. Various animal models have been used to demonstrate that the pericytes in the vasculature of primary tumors limit cancer cell metastasis. For instance, Xian et al. showed that in a genetic model of pericyte detachment from the vasculature (PFGFB^{ret/ret} mice), insulinoma cancer cells were more prone to disseminate to liver, kidney and intestine compared with control mice [11]. In addition, Cooke et al. reported that the deficient pericyte coverage of tumor vasculature is correlated with an enhanced metastatic potential of breast cancer cells [12]. On the other hand, the TME composition, and specifically the presence of recruited BM-MSC, seems to directly affect the dissemination potential of cancer cells [7]. For example, co-transplanting MSC with weakly metastatic breast cancer cells into an orthotopic site increases their dissemination potential to the lungs [4]. This pro-metastatic effect is reversible and short-lived, and is mediated by the paracrine activity of the MSC-derived CCL5/Rantes, which enhances the motility and invasiveness of CCR5-expressing cancer cells. Similarly, co-injected MSC with OS cell lines enhance their lung invasion, through CXCR4 activation and VEGF expression [77, 78]. In addition to this signaling mechanisms, a bi-directional metabolic reprogramming between MSC and OS cells has been reported to explain the OS increased invasiveness. The OS cells induce an oxidative stress state in MSC, stimulating them to produce lactate. The secreted lactate then induces mitochondrial metabolism in cancer cells, favoring their high metabolic demand and promoting motility [79•].

Pericytes recruitment and attachment to the forming vessel is regulated mainly by the endothelial cell secretion of PDGF-B, which binds to its receptor (PDGFR- β), expressed by pericyte progenitors [8, 9]. A positively charged retention motif in the C-terminus part of PDGF-B interacts with negatively charged disaccharide building blocks of heparan sulfate proteoglycans (HSPGs) in the pericellular matrix surrounding endothelial cells. This interaction generates the retention of PDGF-B surrounding the endothelial cell, thus creating a chemoattracting gradient for PDGFR- β -expressing pericytic progenitors close to the endothelium [80]. Modifications of these HSPGs by the secretion of heparanases and sulfatases

have been associated with vascular structural changes in primary tumors and with a propensity to dissemination in various cancers [81, 82].

5) MSC at the target organ: the special case of the BM microenvironment

Skeletal metastases are common complications in various malignancies including melanoma, breast, prostate and kidney cancer, severely undermining the quality of life of patients and negatively impacting the overall prognosis. Despite the advances in knowledge about skeletal metastasis pathophysiology, most of the work has focused on the invasive capability of the circulating osteotropic cancer cell, ignoring the potential contribution of the target organ microenvironment [7]. Stromal elements within the BM microenvironment recently

started to have acceptance as determinants for the invasion of osteotropic cancer cells. For instance, blocking PDGF-B signaling with the multi-target tyrosine kinase inhibitor Sunitinib impairs bone invasion of circulating osteotropic lung cancer cell lines [83]. Some of the anti-invasive effects of Sunitinib have been associated with structural changes in the BM vasculature, including the disruption of the perivascular niche through an increased cell death of BM stromal cells (i.e., BM-MSCs). Despite this anti-metastatic effect, Sunitinib has shown to generate hematologic toxicity in patients, posing some restrictions on its clinical use [84].

Various lines of evidence have suggested that cellular elements within the BM may have a role during the formation of skeletal metastasis. First, during extravasation, the endothelium gets retracted upon interaction with the invading cancer cells, involving the Sdf-1/CXCL12 - CXCR4 molecular axis

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Fig. 1 Participation of MSC as pericytes in various aspects of cancer biology: BM-derived cells of mesenchymal origin (green cells) are recruited to the primary tumor following tumor-secreted signals (e.g., Sdf-1). At the primary tumor, they establish as tumor-associated fibroblasts (TAFs) both in the perivascular space (Pericytic TAFs) and the parenchyma (Fibroblastic TAFs). Later, cancer cells intravasate

guided by pericyte-secreted signals (e.g., Sdf-1) and other cell types (e.g., macrophages, not shown here for clarity). Intravasated cancer cells find the target tissue (e.g., BM and liver), where they again follow an Sdf-1 gradient, and interact with MSC as pericytes via CD146. The multicellular complex then invades the tissue parenchyma

[85]. In the BM, a subpopulation of perivascular cells, the CXCL12 Abundant Reticular (CAR) cells, constitute a rich source of Sdf-1/CXCL1 [30, 86•]. Second, various groups have reported the requirement of a functional pericyte-recruiting PDGFR signaling and the presence of Sdf-1/CXCL12-expressing BM-MSc for an adequate invasion of lung and breast cancer cells into bone [83, 87]. Third, invading cells physically interact with BM stromal cells, and this association affects various biological activities as well as the ultimate fate of cancer cells [7, 88]. All these data pointed to an active participation of stromal components within the BM during cancer cells invasion. However, the precise identity of those stromal components, their relationship with the blood vessels and the location where those physical cellular interactions occur during extravasation were still not fully clear. Uncoupling the vascular components (i.e.: disrupting pericyte-EC interactions) provided information to begin answering those questions. Correa et al. reported that in a genetic model of perturbed pericyte coverage of the vasculature (PDGF-B^{ret/ret} mice), both BM and liver were selectively “protected” from invasion by intra-arterially injected B16F10 melanoma cells [89•]. They showed that as pericytes, BM-MSc are required for an efficient extravasation of melanoma cells. BM-MSc attract (via Sdf-1/CXCL12) and physically interact with (through co-expressed CD146) melanoma cells at the abluminal/perivascular space, initiating the transendothelial migration process. Both molecular axes have been linked with metastatic behavior and poor prognosis in patients with melanoma [90, 91], becoming novel therapeutic targets [92]. This report also underscores the participation of BM-MSc in their perivascular localization as a cellular component of the hematopoietic stem cell niche, during the extravasation of circulating cancer cells into hematopoietic tissues such as the BM and the liver. In that respect, the stem cell niche in the BM has been previously established as a preferred target location for the initial invasion of osteotropic cancer cells [93].

Collectively, vascular components of the target tissue stroma, and specifically BM-MSc as pericytes, participate in the dissemination of circulating cancer cells into the BM. Moreover, their localization in special multi-cellular structures such as the stem cell niche in active hematopoietic tissues provides a new perspective not only in stem cell biology, but also in the analysis of cancer cells engraftment into the homing tissue. Acting as pericytes, BM-MSc unprecedentedly become central orchestrators during cancer invasion, by participating in chemoattraction, adhesion and migration of circulating osteotropic cancer cells into the BM parenchyma. Furthermore, some of the known mechanisms associated with invasion now can be linked to MSc, based on the accumulating literature supporting the expression of some of the participating molecules (e.g., CD146, Sdf-1/CXCL12) by those perivascular cells.

Conclusion

The TME is gaining acceptance as a critical component of tissues during the formation of primary and metastatic tumors. Cellular and structural components of the TME, especially those related to the vasculature, are now linked to key aspects of the tumor biology. With the recent identification of the MSc niche in a perivascular location (as pericytes) and the reports of the direct involvement of BM-MSc in cancer, these adult stem cells and their signaling molecules become recognized as central parts of the TME, thus constituting novel therapeutic targets. In addition, all this information provides evidence for a more comprehensive appreciation of MSc, once perceived exclusively as cellular progenitors.

In addition, to serve as the cellular origin of some bone malignancies (e.g., ES), the strategic localization of MSc as pericytes endows them with active/dynamic roles in cancer biology. These roles are exerted at various steps: 1) the formation of the tumor stroma as they are recruited from the BM to be part of the developing TME; 2) control of the intravasation of cancer cells into the tumor vasculature; 3) regulation of the extravasation of disseminating cancer cells once they find the target organ to initiate a distant metastatic tumor (Fig. 1). Importantly, the effect of the pericyte disruption during intravasation at the primary tumor does not reflect the situation during extravasation at the distant target organ. In the former, the defective pericyte coverage promotes distant dissemination [11], while in the latter, it selectively prevents invasion from circulating cancer cells [89•]. Understanding this differential effect can drive the design of novel therapeutic schemes in which adjuvant interventions are added at the time of removal of the primary tumor to prevent new and to treat existing skeletal metastasis.

Finally, by exploiting their key perivascular location, BM-MSc may play additional roles in other aspects of the biology of skeletal metastasis, such as the quiescence/dormancy of engrafted cancer cells, the angiogenic switch that triggers tumor growth and the local anti-tumor immune response.

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Compliance with Ethical Standards

Conflict of Interest Diego Correa reports a patent pending for Bone Marrow Pericytes/MSc: Gatekeepers Controlling Skeletal Metastasis from Melanoma; and a patent pending for In vivo platform for the identification of controlling elements during the process of metastatic cancer dissemination to target organs.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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