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Journal

Current Cancer Drug Targets, 15(88-98)

Author

Wu, Jian

Publication Date

2015-03-15

Peer reviewed

Involvement of Mesenchymal Stem Cells in Cancer Progression and Metastases

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Abstract: Mesenchymal stem/stromal cells (MSCs) are known to be the helpers for the healing of tissue damage, often referred to as ambulatory cells. However, MSCs are also recruited by cancer cells to similarly aid in tumor growth and progression. In this review, some of the key steps in cancer progression and metastases are described including the various steps in which MSCs participate and may play important roles. MSCs aid in cancer cells' ability to evade immune attack, while promoting tumor angiogenesis, even being counter-acting against chemotherapeutics and other drugs used to fight various cancers. Furthermore, MSCs participate in many of the crucial steps in invasion and metastasis, including stimulating the epithelial-mesenchymal transition (EMT) and induction of stem-like properties that allow cancer stem cells to increase their survivability through the circulation. These steps are described in detail. Differences between circulating tumor cells (CTCs) and cancer stem cells (CSCs) are discussed, along with descriptions of the formation of a pre-metastatic niche, the role of exosomes from both cancer cells and MSCs in metastasis and tumor reseeding (self-seeding). More and more, MSCs are being proposed as a promising tumor targeting drug-delivery tool. In order to fulfill this promise, further understanding of the precise roles that MSCs play in the process of cancer metastases must be achieved, in attempting to create remedies that will improve the outcome of available therapeutics.

Keywords: Cancer metastasis, cancer stem cells, circulating tumor cells, exosomes, mesenchymal stem cells, microenvironment.

INTRODUCTION

The value of mesenchymal stem/stromal cells (MSCs) as a therapeutic agent in regenerative medicine, tissue engineering and as a carrier for anti-tumor therapeutics, is becoming more prominent as research continues to demonstrate advantages such as the intrinsic nature of MSCs to migrate to the tumor bed. Contrastingly, as we learn more about metastasis, the role of non-cancerous stromal cells in the progression of cancer is becoming clearly relevant. Metastases are responsible for about 90% of cancer mortalities thus understanding the mechanism by which this occurs is important for the development of novel therapeutics. Cancer cells metastasize from the primary tumor site through a complex process involving: invasion, detachment, intravasation to lymphatic or blood vessels, travel through the circulation, adhesion, arrest, extravasation and migration into the tissue parenchyma, colonization, and finally development of vascularization and proliferation from micrometastases into macroscopic secondary/metastatic tumors. In some cases, a reseeding of the cells to form metastatic tumors within the same tissue type is favored. The metastatic journey is hazardous and difficult for individual cancer cells, where the vast majority

of circulating tumor cells is rapidly destroyed. Thus, tumorigenic cancer stem cells (CSCs) travel with an entourage of supporting cells and recruited platelets in a sort of embolization of small tumor-cell aggregates for protection. MSCs, the paramedic cells of the body always willing to help, are recruited to play a protective and coordinative role to aid tumor cells in immune escape, call in the cavalry to build new blood vessels (angiogenesis), and play a role in various steps of metastases. In this review, we provide an update on the current understanding of the cellular and molecular mechanisms of metastases with particular attention on the roles of MSCs and what precautions may need to be taken with the evolution of novel MSC-based cancer therapeutics.

MSCs AID IN TUMOR PROGRESSION – UNDER-SCORING IMMUNE ESCAPE, ANGIOGENESIS AND DRUG RESISTANCE

Immune Escape

Even before breaking away from a primary tumor, a cancer cell must veil itself from the immune system – a hallmark of cancer known as immune escape. Abnormal cells, such as malignant cells, are quickly destroyed by the immune system, in particular, by NK cells and major histocompatibility complex 1 (MHC-1) restricted cytotoxic T lymphocytes (CTLs). The perforin/granzyme and the cell death receptor modules used by these cells are modulated by

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regulatory T cells (T_{regs}). There are several putative immune-escape mechanisms conferred by cancer cells themselves [1]. For example, in a zebra model of osteosarcoma, whole genome analysis revealed that the transformed cells had reduced expression of major histocompatibility complex 1 (mhc1ze) genes in contrast to untransformed cells [2], thus allowing the transformed cells to escape from immune recognition. It is evident however, that MSCs play an important role to aid tumors in this function. MSCs are often thought of as ambulatory cells which aid in the wound healing process in part by helping to turn off attacking immune cells and calling in repair cells such as angiogenic ones. For example, MSCs will promote the expansion of T_{regs} and will dose-dependently inhibit mitogen-activated T cell proliferation through the inhibition of cyclin D2 leading to cell cycle arrest at the G0/G1 phase [3, 4]. Tumors, acting as non-healing wounds will elicit a similar action from MSCs recruited to the tumor bed.

It appears that the immunosuppressive behavior of MSCs is induced by inflammatory cytokines, especially within the tumor environment. This was demonstrated by *in vivo* experiments involving the co-injection of MSCs with the mouse prostate cancer cell line RM-1. Secretion of the inflammatory cytokine IL-1 α by the RM-1 cells upregulated the expression of transforming growth factor- β (TGF- β) in MSCs, and led to an increase in tumor incidence [4]. TGF- β 1 secreted by MSCs is implicated in T_{reg} expansion particularly FoxP3-positive T_{regs} , which are well known to have immunosuppressive roles. In a study of breast cancer, granzyme B release by CTLs was significantly reduced in the presence of MSCs. Significant reductions in the responses of NK cells and CTLs were observed with corresponding increases of TGF- β 1 and T_{regs} in the presence (vs. absence) of MSCs and resultant survival and expansion of the cancer cells [3, 5].

Angiogenesis

The ability of MSCs to stimulate angiogenesis is a property being exploited for the treatment of diseases, such as critical limb ischemia [6, 7]. This property has long been similarly exploited by cancer cells for tumor angiogenesis. It appears that tumors may actually take this a bit further, in that MSCs demonstrate a tendency to organize in clusters and to form capillary-like structures, in the presence of cancer cells (e.g. MCF-7 breast cancer cell line) [8]. Furthermore, MSCs secrete various factors that not only attract vasculogenic cells, such as vascular endothelial cells, but also increase their survival and inhibit apoptosis. These secreted factors include interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), and monocyte chemoattractant protein (MCP-1) and act *via* PI3K-Akt-ERK pathways [9]. Such support by MSCs plays a critical role for these vasculogenic cells that may otherwise succumb to the hypoxic tumor microenvironment before they can form the vessels critical to alleviating the tumor hypoxia.

The angiogenic stimulatory nature of MSCs is enhanced within the highly hypoxic and inflammatory tumor microenvironment, which may be due to inflammatory factors interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α). The effects of IFN- γ and TNF- α were demonstrated *in vivo*

in a study where MSCs, pre-stimulated by both factors then injected subcutaneously to BALB/c mice together with colon cancer cells, further enhanced tumor growth and angiogenesis to a greater degree than non-pre-treated MSCs. A similarly enhanced angiogenic effect was observed in the same study in an *ex vivo* hen egg test-chorioallantoic membrane (HET-CAM) assay using conditioned media from IFN- γ /TNF- α -stimulated MSCs vs. conditioned media from MSCs only [10].

Taken together, these observations suggest that anti-angiogenic cancer therapies may be useful in targeting tumor-associated stromal cells, such as MSCs within tumors. MSCs not only help stimulate angiogenesis, but may further help guide invasive cancer stem cells to blood vessels, where they may leave the hypoxic tumor environment and escape to metastasize. This guidance may in part be accomplished through the secretion of the cytokine CCL5/RANTES. CCL5 expression and secretion from MSCs has been shown, by our lab and others, to be increased in the presence of cancer cells (e.g. metastatic breast cancer cell line MDA-MB-231) [11].

Drug Resistance

In addition to conferring increased survivability to angiogenic cells in the hypoxic environment, MSCs may also serve a protective role towards various cancer cells from some standard of care treatments. This has most clearly been demonstrated in leukemia. It is known that MSCs within the bone marrow physiologically support the self-renewal and differentiation of hematopoietic stem cells (HSCs). Malignant hematopoietic cells found to grow in close association with MSCs are similarly privileged by the mitogenic support of MSCs, which also provide protective support against therapeutic drugs. The low levels of asparagine synthetase (ASNS) in acute lymphoblastic leukemia (ALL) cells lend them susceptible to asparagine depletion by therapeutically delivered asparaginase. However, bone marrow MSCs in ALL patients have increased levels of ASNS and have been shown to protect the ALL cells. Experimental ASNS RNA interference prevents this protective capacity, whereas experimentally induced ASNS expression in the MSCs enhances their protective capacity [12]. MSCs have also been shown to protect chronic myeloid leukemia (CML) cells from the imatinib therapy, conferring CML resistance to this medication by reducing caspase-3 activation and modulating anti-apoptotic Bcl-XL expression *via* the CXCL12/CXCR4 axis [13]. The inability of forodesine to decrease peripheral chronic lymphocytic leukemia (CLL) cells in clinical trials, despite very promising pre-clinical data, was found to be due to MSC protection preventing the forodesine-induced 2'-deoxyguanosine (dGuo) triphosphate accumulation and ATP depletion which would have led to CLL apoptosis [14].

The protective properties of MSCs toward cancer cells against therapeutics are not limited to leukemic cells within the bone marrow niche. A promising treatment for ovarian carcinoma is hyperthermic intraperitoneal chemotherapy (HIPEC), which has been demonstrated to be effective in gastric neoplasia. However, bone marrow MSCs and tumor-associated MSCs, which have been shown to play important roles in ovarian carcinoma metastasis, also appear to secrete

the pro-oncogenic cytokine CXCL12, which induces thermotolerance in ovarian cancer cells *via* CXCR4 [15].

METASTASIS

Invasion and the Epithelial-Mesenchymal Transition (EMT)

The metastatic process begins within the micro-environment of the tumor involving invasion, the disruption of the basement membrane by the tumor cells, requiring alterations in cell-cell and cell-matrix adhesion, matrix degradation and cell motility [16]. As a tumor enlarges, hypoxia is a common consequence. Hypoxia together with certain chemoattractants *e.g.* IL-6, CCL2, PDGF, VEGF-A and IGF-1, acts to recruit MSCs towards the tumor bed. Several other secreted factors from a variety of cancer types (breast, prostate, lung, colon, head and neck cancers) are also being studied for their pleiotropic effects on MSCs [17]. TNF- α and IL-1 β released by tumor cells activate V-CAM on the surface of MSCs which helps slow the momentum of recruited MSCs within the tumor microenvironment, where hypoxic signaling is strong. Some of the key signaling molecules activated in MSCs and cancer cells in the response to hypoxia are hypoxia-inducible factors (HIFs). Membrane type 1 matrix metalloprotease (MT1-MMP) activated in the hypoxic environment acts in concert with HIF-1 α to promote the upregulation of 3BP2 (a pleckstrin homology and Src homology 2 domain-containing) adaptor receptor expression. 3BP2 is thought to mediate oncogenic MSC migration [18]. HIFs have been shown to intercede in communication between MSCs and breast cancer cells, with MSCs potentiating HIF expression in the cancer cells and increasing their metastatic propensity. Resulting signaling between MSCs and cancer cells are in part *via* CCL5 secretion from MSCs interacting with CCR5/CD44, and *via* CXCL10 secretion from MSCs interacting with CXCR5 and CXCR3 on the malignant cells [19]. The cancer cells in turn secrete colony stimulating factor 1 (CSF1), thus recruiting macrophages to the tumor. HIF-signaling further results in increased placenta-derived growth factor (PIGF) secretion from the cancer cells, which induces expression of the cognate receptor of PIGF namely VEGFR1, as well as CXCL10 secretion from MSCs. In turn, CXCL10 appears to increase CXCR3 expression on breast cancer cells, in a HIF-dependent manner [20]. Other MSC-derived factors include IL-17B, IL-6, and IL-8 [21], all of which promote mobility and resultant metastasis (*e.g.* to lymph nodes and lungs) [20], summarized in Fig. 1.

Supporting these actions is the tumor stroma. It is now understood that the tumor stroma plays vital roles in the survival, growth and metastatic progression of cancer. Under stressful conditions such as hypoxia, the tumor stroma can be 'activated' and increases its secretion of signaling proteins such as transforming growth factor β (TGF- β), tumor necrosis factor- α (TNF- α), platelet-derived growth factor (PDGF) and hepatocyte growth factor (HGF) [22]. In response to these signals, some cancer cells will further increase their mobility, become increasingly invasive and eventually metastasize. It is presently unclear whether MSC-cancer cell communication occurs before or as a consequence of tumor stroma activation or if both occur

simultaneously as a result of hypoxia. Nonetheless, one of the first steps in metastasis influenced by these soluble factors secreted from tumor stroma involves a change in the cancer cells from an epithelial phenotype to a mesenchymal phenotype, a process known as epithelial-mesenchymal transition (EMT), which confers the ability to invade and metastasize, and promotes stem cell-like properties [23].

MSCs again play a vital role in encouraging this important transition in cancer cell morphology and ability. MSCs have been shown to stimulate cancer cell production of lysyl oxidase (LOX) [21]. In some cancers such as nasopharyngeal carcinoma, LOX is a tumor suppressor, where knockdown of the LOX gene results in tumor growth and its overexpression results to reduced clonogenicity and cell growth [24]. The tumor suppressor activity of LOX appears to be due to the lysyl oxidase propeptide. However, the ability of the active LOX enzyme to oxidize lysine residues in various proteins confers multifunctionality, especially in promoting malignancy and metastasis [25]. An increase in LOX expression, in part due to MSC signaling, is associated with several types of cancer, such as breast, prostate, pancreatic, lung and gastrointestinal cancers [26]. LOX itself is sufficient to enhance metastasis, but also acts within the CD44-TWIST axis [21]. Activation of the transmembrane receptor CD44 on cancer cells (*e.g.* possibly by CCL5 binding to the extracellular domain) results in the CD44 intracytoplasmic domain (CD44-ICD) being cleaved apart from the transmembrane protein and translocating within the nucleus. Inside the nucleus, CD44-ICD is capable of activating HIF-1 α responsive genes by binding to recently discovered DNA consensus sequences, which constitute CD44-ICD response elements in the promoter region of these genes [27]. One of the genes whose expression is turned on by CD44-ICD binding is the LOX gene. Activated LOX will in turn stimulate Twist transcription. Among other actions, Twist1 induces the initiation of EMT, in part by directly repressing the homeobox protein HOXD10, activating ZEB transcription factors, and targeting *miR10b*, a microRNA strongly implicated in metastasis [28-30], thus mediating MSC-triggered EMT [21], as illustrated in Fig. 2.

With the occurrence of EMT, the cancer cells lose their epithelial-like morphology and change to a non-polarized, motile, spindle-shaped cell morphology [31]. Cells undergoing EMT will first lose adhesion to their neighbor through the degradation of E-cadherin and repression of the E-cadherin gene. In its place, vimentin, fibronectin, and N-cadherin will be expressed; whereas β -catenin released from E-cadherin switches its roles from a cell adhesion protein to a transcription factor [31, 32]. The expression of several other genes will be turned on including those encoding VEGF-B, the receptor for VEGF-B, Flt1, and the metalloproteinases MMP-9 and MMP-1. Several transcription factors, such as Slug [31], Snail [33-37], Twist1 [35, 38], and the ZEB family of transcription factors, will also be activated, and their roles are slowly being elucidated [35, 39, 40]. Corroborating the idea of dedifferentiation of cancer cells to take on stem-like properties as cancer progresses, these transcription factors are known regulators of embryonic morphogenesis, yet are highly expressed not only in all cancer cells but specifically in metastatic cells, but also are documented to play various roles in facilitating EMT [32].

Fig. (1). Schematic illustration of various signaling between MSCs and cancer cells, leading to MSC homing to the tumor bed, recruitment of macrophages and metastasis. This signaling cascade supports the invasive and metastatic properties of cancer cells. Hypoxia resulting from an enlarging tumor acts synergistically *via* hypoxia inducible factors (HIF) with chemoattractants such as interleukin-6 (IL-6), CCL2, platelet-derived growth factor (PDGF), vascular endothelial growth factor-A (VEGF-A), and/or insulin-like growth factor (IGF-1) to recruit MSCs to the area. Other molecules like tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) then take over to slow and ensure MSC recruitment through activation of V-CAM. CCL5 secreted by MSCs and binding to CCR5/CD44 on cancer cells in part induces colony stimulating factor 1 (CSF-1) release from cancer cells which helps recruit macrophages.

This suggests that there may be a direct link between EMT and the acquirement of CTC properties as well as additional dedifferentiation to having CSC properties. It would thus follow that MSCs contribute to a microenvironment that

specifically supports circulating CSCs [41], which are able to metastasize and form new metastatic nodules in distant sites [42].

Fig. (2). Mesenchymal stem cells (MSCs) help to trigger the epithelial-mesenchymal transition in cancer cells. By secreting a factor and activating CD44, this causes the intracytoplasmic domain to break away and enter the nucleus. Inside the nucleus, several genes involved in EMT and metastasis are turned on *via* the actions of lysyl oxidase (LOX) to induce a twist basic helix-loop-helix transcription factor (TWIST) cascade involving microRNA *miR10b*, the zinc finger E-box binding homeobox (ZEB) transcription factors, and homeobox D10 (HOXD10) signaling.

Besides *miR-10b*, other microRNAs of note include *miR-373* and *miR-155*. Its precise actions are not yet elucidated but *miR-373* has oncogenic properties regulated by large tumor suppressor 2 (LATS2), and pro-metastatic properties regulated by CD44 [29, 32]. CD44 is one of the major molecules found at the leading edge or invadopodia of invading cancer cells. Specifically its variant form CD44v6 [43, 44], the form found in most metastatic carcinomas, is associated with MMP-9, in a concerted effort to degrade the extracellular matrix, invade the surrounding stroma, and mobilize the cancer cells [45]. Oncogenic expression of the microRNA *miR-155* has recently been shown to be upregulated in hepatocellular carcinoma (HCC) by S100A4, secreted by liver cancer-associated MSCs. This microRNA, *miR-155* activates STAT3 signaling in HCC cells through the down-regulation of suppressor of cytokine signaling 1 (SOCS1). STAT3 signaling consequently promotes the expression of MMP-9 [46], which when coupled with

CD44v6 thus demonstrates an MSC-mediated invasive mechanism (Fig. 3).

Intravasation, Transport and Extravasation

It is not clearly understood how tumor cells enter the bloodstream, however the hydrostatic pressure exerted by the growth of a tumor combined with the abnormality and leakiness of tumor vasculature (blood and lymph vessels) implies a route of least resistance for disseminating cancer cells [31]. The chemoattractant gradient created by CCL5/RANTES secretion from MSCs [11] stimulated angiogenesis within the cancer cell environment, and it may also act as a guide for tumor cells towards vessels into which they may intravasate. Regardless of the route of entry, it is estimated that a malignant tumor may shed 3 to 4 million tumor cells per gram of tumor each day into the blood stream. However, few of these cells will survive and settle to

Fig. (3). Mesenchymal stem cell (MSC) mediation of invasive properties of hepatocellular carcinoma via the upregulation of MMP-9.

Liver cancer-associated MSC secrete S100 calcium binding protein A4 (S100A4) resulting in the upregulation of microRNA *miR-155*, which activates STAT3 through the downregulation of suppressor of cytokine signaling 1 (SOCS1), and leads to increased expression of matrix metalloproteinase 9 (MMP-9). MMP-9 associates with CD44v6 in the liver cancer cells upregulated by microRNA *miR-373*. Thus, CD44v6-associated MMP-9 degrades the extracellular matrix in a highly directed manner at the leading-edge of invasive cancer cells.

form secondary metastatic tumors [47]. Many of the cells are eliminated by the actions of natural killer (NK) cells or other immune cells; whereas many others simply do not survive the shear force of the circulation [31].

Tumor cells average approximately 20-30µm in diameter while the average capillary has an internal diameter of 6-7µm. Yet, most metastases do not result from cancer cells becoming trapped in capillary beds. The cells that do form metastases have long been referred to as CTCs. However, CSCs express various cell surface markers that appear dependent on cancer type, along with the distinct traits of self-renewal and tumorigenesis. The question remains regarding what relationship exists between CTCs and CSCs. It is now suggested, that within the population of CTCs there exist the cancer stem (or stem-like) cells, which may be the actual cells that form metastases. Precise identification of CSCs within CTCs is blurry especially in consideration that cell surface markers, upon which CSCs are defined, may be dynamically expressed. Expression of such CSC markers (e.g. CD44v6) may depend upon the current state of the cells – whether quiescent, invading, travelling through the

circulation or establishing a new colony at a metastatic site. Other distinguishing factors may exist such as differences in the level of expression of metadherin, which aids in the adhesion of cancer cells to the endothelium, and confers an enhanced chemoresistance to the cells [48]. Recent evidence also suggests that autophagy is a prosurvival trait held by CSCs, which may also allow them to travel through much smaller vessels [49-52].

Some organs prone to malignant metastases, such as the liver have discontinuous basement membranes and are very well vascularized. However, selective adhesion to the vascular wall and extravasation of cancer cells also occurs in specific organs and often at sites of inflammation. Additionally, endothelial cells of different organs express unique markers that can be detected by tumor cells. The mechanisms utilized by tumor cells to extravasate between the vascular endothelial cells are thought to be similar to those used by inflammatory cells involving various glycan/selectin/integrin molecule interactions and as suggested above, may involve metadherin as well as epiregulin, MMP1, MMP2 and COX2 [48].

The Pre-Metastatic Niche

Lung metastases are among the most common for several different types of cancers. It was once thought that this might be due to the trapping of cancer cells in the pulmonary microvasculature. However, it is now understood that a premetastatic niche is prepared in anticipation of the arrival of tumorigenic cancer stem/initiating cells. For embryonic and adult stem cells, the differentiation fate is determined by positioning and microenvironment regulated by adjacent cells, matrix proteins and signaling molecules, i.e. a niche. The metastasizing CSCs appear to require a similar sort of niche, formed prior to its arrival. Precisely how the premetastatic niche is formed remains a mystery that is slowly being uncovered. It is now understood that hypoxia at the primary tumor is a potent activator of LOX, which not only signals for cancer cells to undergo EMT when secreted by tumor-associated MSCs [21], but also mediates the recruitment of bone marrow progenitor cells to the premetastatic niche [53]. LOX is an important enzyme in remodeling of the extracellular matrix. In wound healing and fibrosis, LOX acts to cross-link the extracellular matrix proteins, such as collagen and elastin, to stabilize the fibrils in the biogenesis of connective and/or fibrotic tissues [54, 55]. The same cross-linking facilitates premetastatic niche formation. LOX cross-linked collagen IV to which LOX-recruited bone marrow progenitor cells can adhere [53]. For lung metastases, these cells are specifically CD11b⁺Gr1⁺ myeloid progenitor cells (Gr1⁺ cells) that are recruited to the lungs from the bone marrow [56, 57]. Few Gr1⁺ cells are found in the stroma of primary tumors, suggesting specificity for metastatic niche formation. Once in the lungs, the Gr1⁺ cells secrete versican, which promotes the formation of metastatic tumor (discussed below) [57]. Other factors associated with the pre-metastatic niche and Gr1⁺ cells include the proangiogenic factor BV8, MMP9, LOX [53, 56], TGF- β [58] and active myeloid-derived suppressor cells [56, 57].

Soluble factors released by tumor cells, specifically VEGF-A, TGF- β and TNF- α stimulate expression of S100A8 and S100A9 in pulmonary myeloid and endothelial cells, which act to support the adhesion and invasion of metastasizing cancer cells within the lungs [59]. Tissue adhesion and invasion may also be enhanced by bone marrow-derived hematopoietic progenitors expressing the receptor for VEGF-A, VEGFR1/Flt1, which will similarly migrate to premetastatic niche sites and once at the premetastatic site, will express VLA-4 (also known as integrin $\alpha_4\beta_1$). This suggests a possible niche marker of concentrated VEGFR1⁺/VLA-4⁺ cells [60]. A recent explosion in scientific literature has provided insight with recent evidence revealing that exosomes released from CD44v6-positive CSCs and from bone marrow-derived progenitors are key contributors to premetastatic niche formation delivering specific signaling factors [61, 62] possibly to enrich supporting molecules that act similarly to the S100A molecules.

Tumor Exosomes

Not only in cancer, but also in the realm of cellular communication as a whole, extracellular vesicles (EV) have

emerged as an important means by which cells may communicate across large distances within an organism. Extracellular vesicles consist of microvesicles and exosomes, each with varying characteristics. Briefly, microvesicles are generally considered as budding-off from the plasma membrane as vesicles within 100nm to 1.0 μ m in diameter. Exosomes on the other hand are smaller than their microvesicle counterparts, with diameters ranging from 30 to 100nm, deriving from the endosomal compartment through the fusion of intraluminal vesicles of multivesicular bodies, with the plasma membrane [63]. Both types of EV therefore carry, in their membranes or in their soluble fractions, some characteristics of the plasma membrane of the cell from which they originate. An in depth description is beyond the scope of the current review, however, an example of this in cancer is the transmembrane molecule CD44v6 expressed on metastasizing cancer cells [43, 64, 65]. In the case of pancreatic adenocarcinoma, CD44v6 carried by tumor exosomes in their soluble matrix appears to play a role in premetastatic niche preparation by modulating cells of the organ in provision for tumor cell arrival and embedding [44, 64].

The tendency of certain cancer types to metastasize to particular regions (*e.g.* lung, liver, brain or bone) and the ability for cancer initiating cells to find the premetastatic niche may therefore at least in part be explained by tumor exosomes. This finding has come to light with the discovery that exosomes contain mRNA, miRNAs and signaling molecules, along with advancing technologies to isolate and study them [44]. MSCs may play a part in communicating with tumor exosomes and/or helping the cancer cells find these exosomal-prepared niches. Furthermore, exosomes from tumor-associated MSCs have been found to have differentiated miRNA expression compared to non-cancerous MSCs in such diverse cancers as multiple myeloma and gastric cancers, with both types facilitating malignant progression [66, 67]. For example in gastric cancer, both cancer cell and tumor-associated MSC exosomes contain upregulated levels of miR-214, -221 and -222, compared to adjacent non-cancerous MSCs. Levels of these miRNAs correlated with lymph node metastases. Furthermore, gastric cancer-associated MSC exosomes appeared to be able to deliver miR-221 to the same cancer cells, promoting their proliferation and migratory ability [67]. A similar effect was observed in a breast cancer cell model whereby exosomes generated from MSCs stimulated migratory activity in the non-metastatic MCF-7 breast cancer cell line through activation of the Wnt signaling pathway [68, 69], in a manner comparable to that observed with MSCs isolated from primary breast cancer tissue.

The Mesenchymal-Epithelial Transition, Colonization, Reseeding, and Proliferation

Once the circulating CSCs arrive at its metastatic niche, they will again shed their mesenchymal phenotype and transdifferentiate back to an epithelial-like state. Until recently, little was known about the actual process of mesenchymal-epithelial transition (MET), a necessary step in the CSC's ability to take root, start dividing and forming a metastatic tumor at a distant site. In fact MET was more of a hypothesis until a recent discovery that MET relies on

crosstalk between the primary tumor, the metastatic niche and hematopoietic stem cells that set up the metastatic niche prior to arrival of the cancer cells [57]. This crosstalk is heavily dependent on the protein versican, which has for many years been associated with cancer progression although its precise role was only recently elucidated. Versican, secreted by myeloid progenitor cells within the metastatic niche will mediate MET and attenuate phospho-Smad2 levels in the cancer cells resulting in increased cell proliferation [57]. The accelerated proliferation caused by versican in metastatic niche at least in part facilitates metastatic tumor formation. It is known that 90% mortalities result from cancer metastases rather than the primary tumor [32, 58].

An interesting phenomenon is that sometimes CTCs may recolonize back to their original tumor organ in addition to forming a secondary tumor elsewhere. This process is termed as tumor reseeded or “self-seeding”, and appears to be a property of aggressive metastatic breast cancer, colon cancer, melanoma and HCC cells that have a metastatic propensity or tropism to bone, lung, or brain tissues, etc. but instead seed back to their primary tumor tissue [42, 70]. Such reseeded results in accelerated tumor growth since there is a selective force for more aggressive cancer cell populations than for the bulk population of the primary tumor [70]. Two models are proposed to explain this phenomenon in which there is primary seeding or secondary seeding. In primary seeding, CTCs that enter into the circulation return to the primary tumor. In secondary seeding, a distant metastasis is formed first and progeny CTCs shed into the circulation from this secondary tumor then implant and repopulate within the original organ or primary tumor. Mathematical modeling suggests that the latter model of secondary seeding is several orders of magnitude more likely and thus metastases may actually be drivers of primary tumor growth [71]. Indeed it has been demonstrated that ‘reseeded’ primary tumors have upregulated cytokines (e.g. IL-6, IL-8) that are involved in various processes including extravasation and angiogenic support [72]. Although not yet elucidated, we hypothesize that it is likely that MSCs play a role in favoring the reseeded (or self-seeding) of these cells and/or in supporting their colonization and growth once within the primary tumor site, perhaps by providing various differentiation and mitogenic signaling factors, and chemoattractants for other supporting cells. This role of MSCs will be of interest in investigations into understanding the molecular regulation of tumor reseeded.

CONCLUSIONS AND PERSPECTIVES

Because cancer cells will recruit MSCs to the bulk tumor environment, and may follow chemoattractant gradients provided by MSCs towards vasculature where they can escape into the circulation, it is very plausible that engineered MSCs may be used therapeutically to block cancer metastases. For example, besides activating MSCs, specific members of the IL-6 family such as cardiotrophin-1 (CT-1), may play dramatic roles in metastasis and could represent therapeutic targets for MSC-delivered gene silencing. In a mouse model of colon cancer with hepatic metastases, CT-1-null mice rejected hepatic engraftment of

colon cancer cells (MC38), which gave rise to lethal hepatic tumors in their wild-type mouse counterparts [73]. Applications for MSC-delivered therapy may take advantage of the tumor-specific expression of molecules and use their promoters to drive expression of pro-apoptotic molecules. Alternatively, a complementary application may involve the knockdown of MMP-9 or -1 expression with siRNA delivered by MSCs or targeted MSC exosomes to prevent invasive migration. An example of such a strategy was demonstrated in a rat model using exosomes from bone marrow MSCs transfected to express miR-146b. The harvested MSC exosomes contained and effectively delivered miR-146b to glioma tumors with intra-tumor injection, resulting in a significant reduction in glioma growth [74]. Similarly, siRNAs could conceivably be MSC-delivered to attenuate the activation of transcription factors involved in the EMT transdifferentiation of cancer cells, such as Slug [31] and Snail [33] which repress E-cadherin transcription, and Twist1 [38].

Proposed cancer therapies in pipeline now include exosomes, antibodies and small molecules to inhibit tumor growth and kill cancer cells. Because of the innate recruitment of MSCs to tumor sites and interactions of MSCs with cancer cells, the utility of MSCs as a delivery vehicle confers a great advantage over administration of these and other therapeutic molecules on their own (or in micelles) intravenously. This is because even the specificity of antibodies has limitations possibly encountering similar antigens systemically, and the tumor may be inaccessible for direct injection to the tumor site. Despite this great advantage, because of the significant contribution of MSCs to tumor growth, progression, dissemination and metastasis, a thorough understanding of the interplay of MSCs with the metastatic process is a prerequisite before safe and effective MSC-delivered therapeutics can be clinically applied for cancer therapies.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Our work on MSC therapy for cancer and other diseases is funded by the NIH Director's transformative award 1R01GM099688 (Nolta) and philanthropic donors including the Levy family. Dr. Nolta is funded through the California Institute for Regenerative Medicine (CIRM) and program start-up funding from the Deans Office, UC Davis School of Medicine. Dr. Chang is a scholar of the Howard Hughes Medical Institute Integrating Medicine into Basic Science Program at the University of California Davis (HHMI-IMBS). Dr. Wu is supported by the Natural Science Foundation of China (NSFC #81272436) and the Fudan University Starting Fund.

LIST OF ABBREVIATIONS

ALL	=	acute lymphoblastic leukemia
ASNS	=	asparagine synthetase
CSF1	=	colony stimulating factor 1

CSCs	=	cancer stem cells
CT-1	=	cardiotrophin 1
CTCs	=	circulating tumor cells
CTL	=	cytotoxic T lymphocyte
EMT	=	epithelial mesenchymal transition
HCC	=	hepatocellular carcinoma
HET-CAM	=	hen egg test-chorioallantoic membrane
HSC	=	hematopoietic stem cells
IFN- γ	=	interferon- γ
IGF-1	=	insulin-like growth factor-1
IL-6	=	interleukin-6
LOX	=	lysyl oxidase
MCP-1	=	monocyte chemoattractant protein
MET	=	mesenchymal-epithelial transition
MHC-1	=	major histocompatibility complex 1
mhc1ze	=	major histocompatibility complex 1 gene
MSCs	=	mesenchymal stem/stromal cells
MT1-MMP	=	membrane type 1 matrix metalloprotease
NK cells	=	natural killer cells
PDGF	=	platelet-derived growth factor
PIGF	=	placenta-derived growth factor
SOCS1	=	suppressor of cytokine signaling 1
TNF- α	=	tumor necrosis factor- α
VEGF	=	vascular endothelial growth factor
VLA-4	=	very late antigen 4 (also known as integrin $\alpha 4 \beta 1$)

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Received: October 28, 2014

Revised: December 28, 2014

Accepted: January 13, 2015