Title

Mesenchymal Stem Cells as a Carrier for Targeting Anti-Tumor Therapeutics

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18 Mesenchymal stem cells as a carrier for tumor-targeting therapeutics

Abstract Current chemotherapy is not tumor-selective and gives rise to severe adverse effects for patients. Mesenchymal stem cells (MSCs) exhibit a unique tumor-homing property and could be used as a drug carrier for targeting tumor therapy. The tumor-homing property of MSCs depends on the hypoxia and inflammatory status in the tumor, and is modulated by factors such as vascular endothelial growth factor (VEGF), hypoxia-inducible factor-1α (HIF-1α) or other cytokines released from tumors. MSCs may be isolated from umbilical cord blood or adipose tissues, and are readily engineered for carrying therapeutics, such as oncolytic adenovirus, specific cytotoxic molecules, nucleotides, prodrugs cytokines or antibodies, or to produce therapeutic molecules within a tumor site. The most promising therapeutics include blockers for VEGF, prodrugs (*e.g.* ganciclovir), oncolytic adenovirus, thymidine kinase, and pro-apoptotic "TRAIL". The efficacy of these bio-engineered MSCs has been evaluated in animal models of pulmonary, breast, gastrointestinal, and pancreatic cancer xenografts grown in immune-deficient mice, and their safety has been shown in some early phase human trials, but they have yet not been moved to later phase clinical application. Although these novel approaches are promising, MSCs may have some risks for cancer patients since MSCs are found to be immunosuppressive in tumor sites, are pro-angiogenic, and in some cases may promote tumor growth. Therefore, whether bio-engineered MSCs will be a useful therapeutic vehicle depends on the property of the specially engineered cell population, tumor types and locations, as well as the time and route of administration of MSCs-based therapeutics. This chapter discusses approaches to utilize MSCs' tumor-homing properties for improving current cancer therapy.

18.1 Introduction

Mesenchymal stem cells (MSCs) are a promising cell therapy in a wide variety of tissue injuries and disorders, whether acting directly, as in repair of bone, tendon and cartilage; indirectly as an immune modulator or revascularizing agent; or as a biocarrier for drugs, peptides, proteins, or other gene products. Preclinical studies in neurodegenerative diseases [1], cardiovascular diseases [2, 3], autoimmune diseases [4, 5] and others have allowed for this promise to be quickly brought to fruition as clinical trials testing the utility of MSCs for targeting diverse diseases are currently ongoing. This chapter provides an overview of how MSCs are therapeutically useful in targeting malignant tumors. Various cell intrinsic and environmentally responsive properties make MSCs highly attractive for therapeutic uses, especially in the realm

of cancer. These properties are described in detail in this chapter along with how MSCs could be utilized or manipulated to suppress tumor growth and prevent deadly metastases.

18.2 Enhanced angiogenesis as a target for tumor therapy

A key feature of malignancy is uncontrolled cell division. For a tumor to grow beyond a diameter of 1 to 2 mm, cancer cells need to interact with and gain support from stromal tissue including vascularization. In an early investigation describing the tumor as a wound that does not heal, it is pointed out that the dense firm nature of many solid tumors is due largely to collagenous stroma (Fig. 18.1), which in some cases may account for more than ninety percent of the total tumor mass [6]. Due to the hypoxic environment surrounding the growing mass, tumors often behave like wounds that activate the intrinsic healing response and induce the surrounding stroma to attract inflammatory cells, fibroblasts, and angiogenic cells, similar to some of the events in physiological wound healing. In this way, the tumor also becomes vascularized *via* angiogenesis, which plays an essential role in tumor growth and metastasis [7, 8]. Therefore, it appears that anti-angiogenesis should be an excellent target for cancer therapeutics and indeed has been a major arena for drug development in the past decade. However, there have been some major shortcomings and

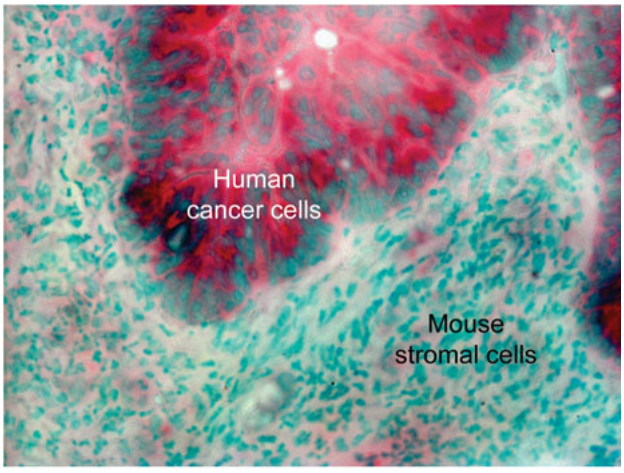

Fig. 18.1: Stromal involvement in a malignant tumor. Shown here is a representative section of xenografted human colon cancer cells (red) that have developed to a large subcutaneous tumor in a mucopolysaccharidosis type VII (MPSVII) mouse model demonstrating that a large amount of non-cancer cells (non-red) may be part of a solid tumor. Histochemical staining was based on the presence (red – human cancer cells) or absence (non-red – mouse stromal cells) of glucoronidase. 200X.

how to deliver effective therapeutics selectively to tumor parenchyma and to avoid affecting normal cells is a long-term effort.

Under physiological conditions, angiogenesis is a tightly controlled process balanced by proangiogenic and angiostatic factors, as well as cell-cell and cell-extracellular matrix interactions. Vascular endothelial growth factor (VEGF)-A was identified as the primary proangiogenic factor during the 1990s [9], with basic fibroblast growth factor as a close second [10]. Oncogenic mutations resulting in the increased Ras expression also lead to the upregulation of VEGF-dependent angiogenesis [11]. VEGF-A is known to increase vascular leakage, and is therefore a vascular permeability factor, playing important roles in the inflammatory process [7, 12]. Rather than well-organized structures, tumor neovasculature is malformed with tortuous and leaky vessels, due to little adhesion between endothelial cells [13, 14]. The subsequent leakage is due to high levels of VEGF-A released by tumor cells and surrounding fibroblasts stimulated by numerous growth factors such as epidermal growth factor (EGF), TGF-α, TGF-β, keratinocyte growth factor (KGF), insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and hypoxia inducible factor-1 (HIF-1) [7]. The leaky tumor vasculature allows fibrinogen molecules in the plasma to come in to contact with cancer cells and form large fibrin strand bundles, which may further develop into an immense collagenous stroma that helps form or reinforce tumor stroma.

In addition, VEGF-A collaborates with cytokines, such as interleukin-4 (IL-4) and IL-10, in the conversion of M2-polarized macrophages into tumor-associated macrophages (TAM), which promote immunosuppression and tissue remodeling to allow for invasion and metastasis [15]. At the same time, many types of cancer cells continuously release high quantities of the mitogen PDGF, which may attract more mesenchymal cells, such as fibroblasts, macrophages, smooth muscle cells and endothelial cells, to the surroundings of the tumor. Some of these key molecules within tumor angiogenesis may prove to be useful in engineering MSCs for anti-angiogenic therapeutics in cancer therapy.

18.3 Why current therapies are not effective enough

Cancer was responsible for one in every eight deaths worldwide in 2011. Moreover, incidences of certain cancer types are increasing. For example, in the United States breast cancer is the most common and second most lethal type in women. In Korea, a similar scenario arose in 2002 and has remained, with breast cancer becoming the most prevalent type of malignancy in women. Certainly there is a great need to better understand the oncogenesis of these cancers and to develop better therapeutics, which is precisely what physicians and scientists hope to achieve with the use of MSCs.

Radiation and chemotherapy remain the major treatment options for patients who are contraindicative for surgical resection. Nonetheless, these therapies increase dis-

comfort and morbidity, and may be ineffective against tumor-initiating/cancer stem cells, yet cause toxicity or killing of normal cells. Tumor-initiating cancer stem cells are now thought to be the culprits of metastasis, which are responsible for the vast majority of oncogenic fatalities [16]. However, it is important to note that there are currently few pharmaceutics on the market to target these cells. Similarly, in most cases the high incidence of mortality in patients with pulmonary malignancies (whether lung cancer or pulmonary metastatic diseases) is due to a lack of ability to deliver targeted therapeutics. On the other hand, a wide range of pharmaceutics therapeutically target tumor angiogenesis, which is an excellent example of how current therapies, although they are the best available, may still be insufficiently effective.

18.3.1 Shortcomings of current anti-angiogenic pharmaceuticals

As described above, antitumor angiogenesis is an intuitive target for cancer therapeutics, and this therapy has been used for various types of malignancies [17]. It is clear that VEGF-A plays a key role in angiogenesis and has been a primary target for anti-angiogenic therapies due to its abnormally high expression in most human malignancies and association with poor prognoses [18]. FDA-regulated Phase IV clinical trials continue even after therapeutics reach the market. Although preclinical studies aiming to inhibit the VEGF-A pathway have demonstrated decreased tumor growth and have moved to clinical application, recent clinical observations are demonstrative of the limited efficacy of these therapies. For example, bevacizumab (commercially known as Avastin®), a recombinant humanized monoclonal antibody specifically targeting VEGF-A, is a current standard of care; but it is among pharmaceuticals that have led to increased morbidity but have not increased overall patient survival. Ranibizumab, also targeting VEGF-A, is a monoclonal antibody. Ramucirumab is a monoclonal antibody against the VEGFR-2 receptor, whose primary ligand is VEGF-A. Aflibercept is an anti-angiogenic agent designed to target both VEGF-A and -B as well as PDGF. Aflibercept has been shown to inhibit VEGF-induced angiogenesis in preclinical laboratory and animal models, and promotes progression-free survival in Phase III clinical trials; but again it does not positively affect overall survival [14]. Moreover, targeting the angiogenic cascade are several tyrosine kinase small-molecule inhibitors, such as ramucirumab, ranibizumab, sunitinib and pazopanib [14]. With such a multitude of various antitumor angiogenic agents and the lack of extension of overall survival, it is unfortunately clear that these agents are not as effective as had been anticipated [19].

It is now suggested that anti-angiogenic agents may actually stimulate or potentiate invasive and metastatic properties [20]. Cancer stem cell enrichment within a tumor is driven by the Wnt signaling pathway activated *via* the Akt/β-catenin pathway, which is stimulated by hypoxia-inducible factor 1α (HIF-1α) during hypoxia caused by anti-VEGF agent treatment (Fig. 18.2). Hypoxia is also a potent inducer of the epi-

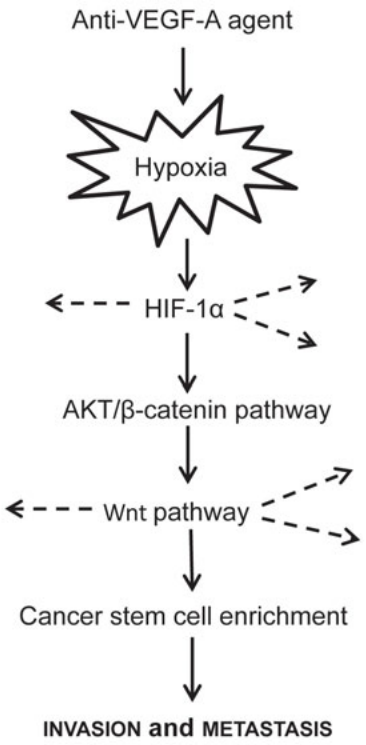

Fig. 18.2: Anti-VEGF-A therapeutics may induce hypoxia. A schematic flow chart illustrates the possible unwanted consequences of anti-VEGF-A which may induce hypoxia within a tumor, ultimately resulting in an enrichment of cancer stem cells that are believed to be more invasively aggressive and metastatic.

thelial-mesenchymal transition (EMT), a sort of transdifferentiation of cancer cells with an epithelial-like character, in which they acquire a motile, more mesenchymal-like phenotype [21]. EMT is thought to be the initiation of metastasis and is initiated by upregulation of metalloproteinase release by tumor cells. Matrix metalloproteinases (MMPs), acting to disrupt the basement membrane, may activate HIF-1α, and promote intravasation [22, 23]. Inhibition of these enzymes has been considered as an anti-metastatic target, and MMP inhibitors could be delivered tumor-specifically with the use of MSCs. Although VEGF-D is physiologically involved in lymphangiogenesis along with VEGF-C, higher levels of VEGF-D expression have been observed with anti-angiogenic therapy; and are now thought to be predictive of resistance to anti-angiogenic agents [24]. The role of VEGF-D in promoting tumor angiogenesis is not currently known, however it appears to be involved in the process especially in the absence of VEGF-A, for example, following the removal of VEGF-A by anti-angiogenic agents [24]. Yet another angiogenic player is placental growth factor (PlGF) [25].

Although PlGF expression may not be augmented in all tumors, several studies now implicate that PlGF is so abundant in the angiogenic switch in neoplastic cells, that it has quickly become a prognostic marker in some cancers [14, 26]. Furthermore, angiopoietin may also be involved in tumor angiogenesis since its receptor, Tie-2, is overexpressed in tumor vasculature, which is also associated with poor prognoses [27] and is one of the targets of early tumor therapeutic engineered MSCs [28].

Thus, one of the primary problems in targeting angiogenesis as an anticancer therapeutic is that there exists tremendous redundancy in the process. With conventional therapeutics patients may find themselves with the arduous task of having to be administered a large multitude of various drugs to target the critical process of angiogenesis in tumor growth, let alone other processes in addition, such as proliferation, cell cycle progression, apoptosis, migration, invasion and metastasis.

18.4 Why mesenchymal stem cells would be useful for tumor targeting

18.4.1 The tumor-homing properties of MSCs

Physiologically, MSCs are thought to contribute to the maintenance of stromal and connective tissues in organs remote from the bone marrow – a function that gives purpose to their highly proliferative attribute. In wounds where tissue damage is being repaired and cell turnover is thus increased, MSCs may be engrafted in and become part of the tissue [20]. This property probably explains Wagner's observations in patients with another type of chronic inflammatory disorder, epidermolysis bullosa. These patients received allogeneic bone marrow transplants and showed similar engraftments of the allogeneic cells in blistering areas of the epidermis [29]. This property also in part explains the strong tropism of MSCs to tumors due to their high resemblance to wounds. The innate ability and actions of MSCs to home to sites of hypoxia and inflammation [30], including tumors, have been extensively investigated by many groups. We transduced MSCs to constitutively express green fluorescent protein (GFP) to track migration to the tumor bed following intravenous (tail-vein) injection in an immune deficient mouse xenograft model of pancreatic cancer. MSCs migrated to metastatic tumors as demonstrated by the localization of these green cells in tumor parenchyma (Fig. 18.3).

The precise mechanisms through which MSCs are recruited to sites of inflammation and hypoxia are not fully understood. Nonetheless, several pathways have been implicated to play roles in the enhanced migratory signaling of MSCs trafficking. Some of the postulated mechanisms are equally responsible for the recruitment process in hypoxic states as well as in the inflammatory process. Tumors exhibit both hypoxia and release many similar cytokines as are released in the inflammatory process in areas of injury/wounds although the complex interplay between MSCs

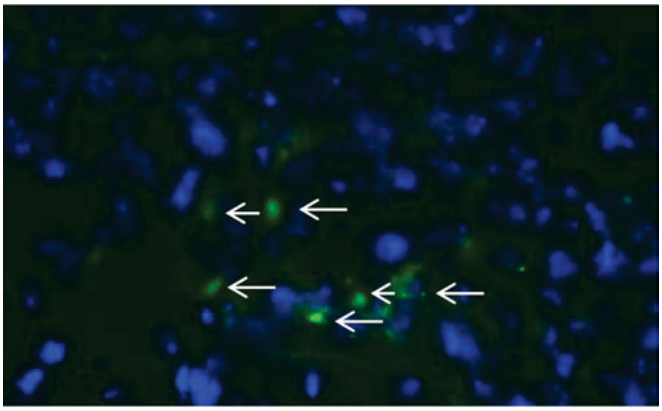

Fig. 18.3: Mesenchymal stem cell (MSCs) home to the tumor bed. A representative section of a xenografted human breast cancer (MDA-MB-231) tumor in a mouse model in which human mesenchymal stem cells (MSCs) expressing GFP (green) were administered by tail-vein injection and migrated intrinsically to the tumor site. The visualization of MSCs in the xenograft tissue indicates the tumor-homing character of these MSCs. 200X.

and tumors through various cytokines is not yet fully understood. The schematic illustration in Figure 18.4 highlights some of the plausible signaling mechanisms leading to the homing of MSCs to the tumor environment. MSCs trafficking towards hypoxic regions is enhanced by chemoattractants such as IL-6, monocyte chemotactic protein-1 (MCP-1), PDGF and VEGF-A (which act synergistically), and insulin-like growth factor-1 (IGF-1), which are released from areas of injury and inflammation, as well as tumor cells. Secretion of IL-6 from cancer cells is especially upregulated by hypoxia, which may occur as the tumor outgrows its vascularity, or as a consequence of anti-angiogenic therapies as discussed earlier. IL-6 is a cytokine, which normally plays a role in the immune response and inflammation, in part as a result of hypoxic conditions, and acts in a paracrine fashion to recruit and activate MSCs. MSCs recruitment and activation is achieved through the upregulation of the STAT3 and MAPK signaling pathways, both of which enhance MSCs migration as well as their survivability. Thus, both the STAT3 and MAPK pathways play critical roles in the ability of the MSCs to adapt to the hypoxic environment [31].

Both TNF-α and IL-1β released from cancer cells activate V-CAM-1 on the surface of MSCs helping slow the migration of MSCs. Once in an area of hypoxia, the hypoxia itself stimulates HIF-1α in MSCs and appears to mitigate the activity of GTPases, for example decreasing the active form of the GTPase RhoA, which further results in a slow-down of MSCs migration once within the hypoxic environment [32]. However, MSCs may not have arrived at their oncogenic location just yet. The hypoxic environment furthermore activates upregulation of membrane type 1 matrix metalloprotease (MT1-MMP) in MSCs. Activated HIF-1α enters the nucleus and binds to its regulatory element on the 3BP2 promoter (P3BP2). MT1-MMP acts in concert with HIF-1α

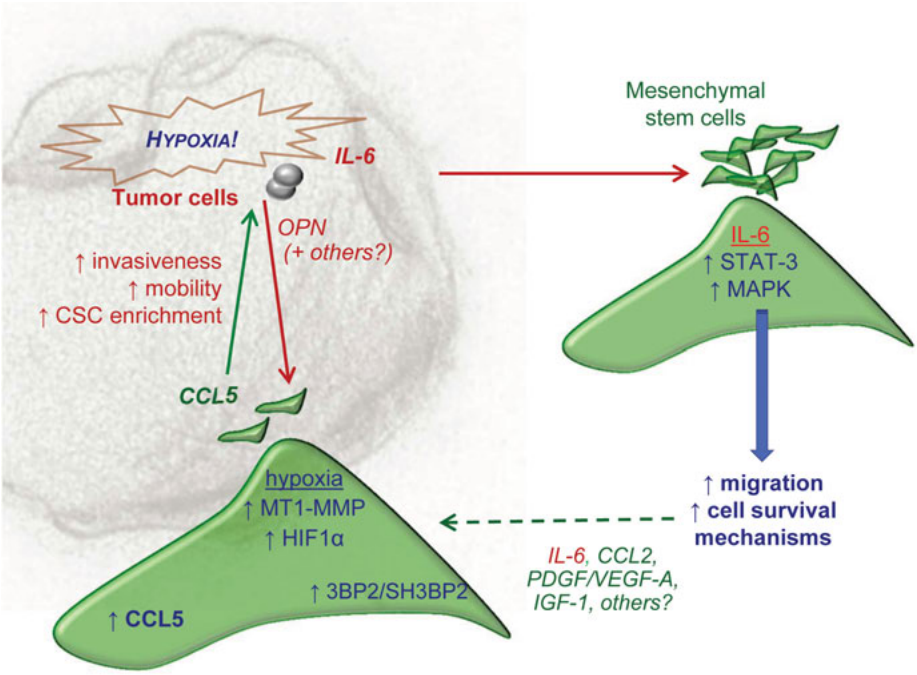

Fig. 18.4: Schematic illustration of the mechanisms leading to the migration of mesenchymal stem cells (MSCs) towards the hypoxic environment within a malignant tumor. Under hypoxic stress, some tumor cells will release IL-6 which will act on distant MSCs, activating the STAT-3 and MAPK signaling pathways which prime the MSCs for migration and increase cell survival mechanisms they will need within the hypoxic tumor environment. The MSCs migrate towards the tumor (green dashed line) following the cues of inflammatory molecules and chemoattractants released from the tumor site, such as IL-6, MCP-1, PDGF, VEGF-A, IGF-1, or others. Cytokines within the hypoxia environment stimulate MSCs to increase their expression of the matrix metalloproteinase (MT1-MMP) and hypoxia inducible factor-1 alpha (HIF-1α). HIF-1α will enter into the nucleus and bind to the P3BP2 promoter to induce genes for enhanced mobility and migration in both MSCs and cancer cells. Together with osteopontin (OPN) and possibly other molecules released from tumor cells, HIF-1α will additionally act within MSCs to increase expression of the chemokine CCL5. CCL5 released by MSCs acts on cancer cells to increase invasive actions, mobility, proliferation and enrichment of cancer stem cells (CSC) and may also act as a gradient that migratory cancer cells follow towards the MSCs and vasculature through which they may metastasize. The signaling molecules incorporated here are by no means comprehensive and much of the precise mechanisms remain to be elucidated. Red – molecules released by or actions stimulated within cancer cells. Green – molecules released by or actions of mesenchymal stem cells. Blue – molecules acting on or gene expression increases or actions stimulated in both mesenchymal stem and cancer cells. Acronyms (approximately clockwise): IL-6 = interleukin 6, STAT-3 = signal transducer and activation of transcription 3, MAPK = mitogen-activated protein kinase, PDGF = platelet-derived growth factor, VEGF-A = vascular endothelial growth factor A, IGF-1 = insulin-like growth factor 1, MT1-MMP = membrane type 1 matrix metalloproteinase, HIF1α = hypoxia inducible factor 1 alpha, OPN = osteopontin.

to promote the upregulation of 3BP2 expression. Although not fully characterized in MSCs, 3BP2, similar to IL-6, is known to play an endogenous role as an immune response adaptor protein that regulates the differentiation of leukocytes and activates their motility. 3BP2 is similarly capable of stimulating MSCs migration, demonstrating a consistent mechanism of 3BP2 action on the role of motility in leukocytes as well as MSCs [33]. It is plausible that MSCs here are switching from an analogous "having taken the water way to the area of their destination" to now "walking the remainder of the way".

Understanding these mechanisms is important to fully take advantage of, while not disturbing, the tumor-homing property of MSCs, in the most efficient manner. These MSCs may be used as vehicles for exosomal delivery of pharmaceuticals, or engineered to express suicide-inducing transgenes of gene products that will halt metastatic communication between tumor-initiating cancer cells and the metastatic niche.

18.4.2 MSCs as a diagnostic tool

One technique with promising clinical utility is currently being developed, which involves MSCs labeled with biocompatible superparamagnetic iron oxide nanoparticles to track the homing of the MSCs to primary tumors as well as to multiple metastatic pulmonary tumors, at very low cell numbers [34]. The nanoparticles generate a local magnetic field perturbation exhibited as a localized hypointensity at a cellular level using magnetic resonance imaging. This application in humans would have great value in detecting possible mini- or micro-metastases that would otherwise be clinically undetectable. Given the high mortality rate due to metastases of tumor cells, MSCs for such diagnostic as well as therapeutic uses are promising for clinical applications. Identified micro-metastases may be operable, however for other types of tumors, there is a clear lack of therapeutics that can directly target them such as pulmonary malignancies (whether lung cancer or pulmonary metastatic diseases) resulting in high incidences of mortalities and poor survival. Therefore, MSCs as a biocarrier to deliver targeted therapies to pulmonary tumors in addition to detection-oriented nanoparticles would be very valuable.

18.4.3 Antitumor effects of unmanipulated MSCs

It has been observed that MSCs homing to the tumor bed may cause growth inhibition and abolishment. This has been demonstrated in a Kaposi's sarcoma murine model in which bone marrow-derived MSCs were administered intravenously, homed to the tumor, and retarded growth [35]. Growth of breast carcinoma (MDA-MB-231), ovarian carcinoma (TOV-112D), and osteosarcoma (MG-63) cells has been inhibited

by extracts from MSCs isolated from Wharton's jelly where all three cancer cell lines exhibited cell shrinkage, apoptotic blebbing and vacuolations, as well as inhibition of migration [36]. In another study, Wharton's Jelly-derived MSCs were shown to also cause regression of mammary carcinomas in a rat model after intratumoral injection. Similarly, nonengineered human umbilical cord-derived MSCs were administered intravenously in a xenografted rat model of human breast carcinoma (MDA-MB-231), and homed to lung metastases where a reduction in tumor burden was subsequently observed [37]. How these MSCs are acting is not understood although it has been postulated that the MSCs isolated from human umbilical cord blood secrete the molecule dickkopf (DKK1), which is a negative regulator of Wnt signaling [38]. The canonical Wnt/β-catenin pathway, which is critical in development, is critical in tumorigenesis, thus secretion of DKK1 results in a suppression of the Wnt pathway, in turn inhibits cancer cell growth. In addition, co-culture of glioma cells with MSCs reduced PDGF release from glioma cells, which may be responsible for the suppression of angiogenesis [39].

All of these antitumor effects by unmanipulated MSCs must be interpreted carefully. The efficacy appears to be strongly dependent on cancer type as well as the source of MSCs. Bone marrow-derived MSCs may have a negative effect on certain sarcomas, but also have the undesirable opposite effects on carcinomas, including participation in the formation of the tumor microenvironment and metastatic niche, promotion of tumor growth and aiding in metastases. However, MSCs isolated from various human umbilical cord tissue or human umbilical cord blood may have suppressive effects on some types of carcinomas, such as breast and ovarian. Further investigations remain to reveal how cancer type-specific these effects are and whether the mechanism of action is mediated directly through cell-cell contact communication, *via* various secreted signaling molecules, or possibly by exosomal communications.

18.4.4 Vesicular communication of MSCs: How MSCs can be used as a drug-delivery vehicle

Exosomes have been identified as vesicular carriers for intercellular communication and are increasingly being found to play vital roles in the information transfer between cells. Exosomes are 40–100nm diameter vesicles (with a density in sucrose of 1.13-1.19g/ml and sedimentation at 100,000g) having a similar topology as a cell and containing a wide array of biologically active molecules. They are formed through the fusion of multivesicular endosomes with the plasma membrane, and released by most cell types [40]. MSCs, sometimes described as ambulatory cells, are of no exception. Time-lapse video recording of MSCs reveals that MSCs are highly active in culture and will crawl right up against neighboring cells as they travel along, appearing to probe them. This includes cancer cells, as demonstrated *in vitro* in Figure 18.5. In most cases cells will leave small exosomal vesicles that MSCs may pick up or MSCs

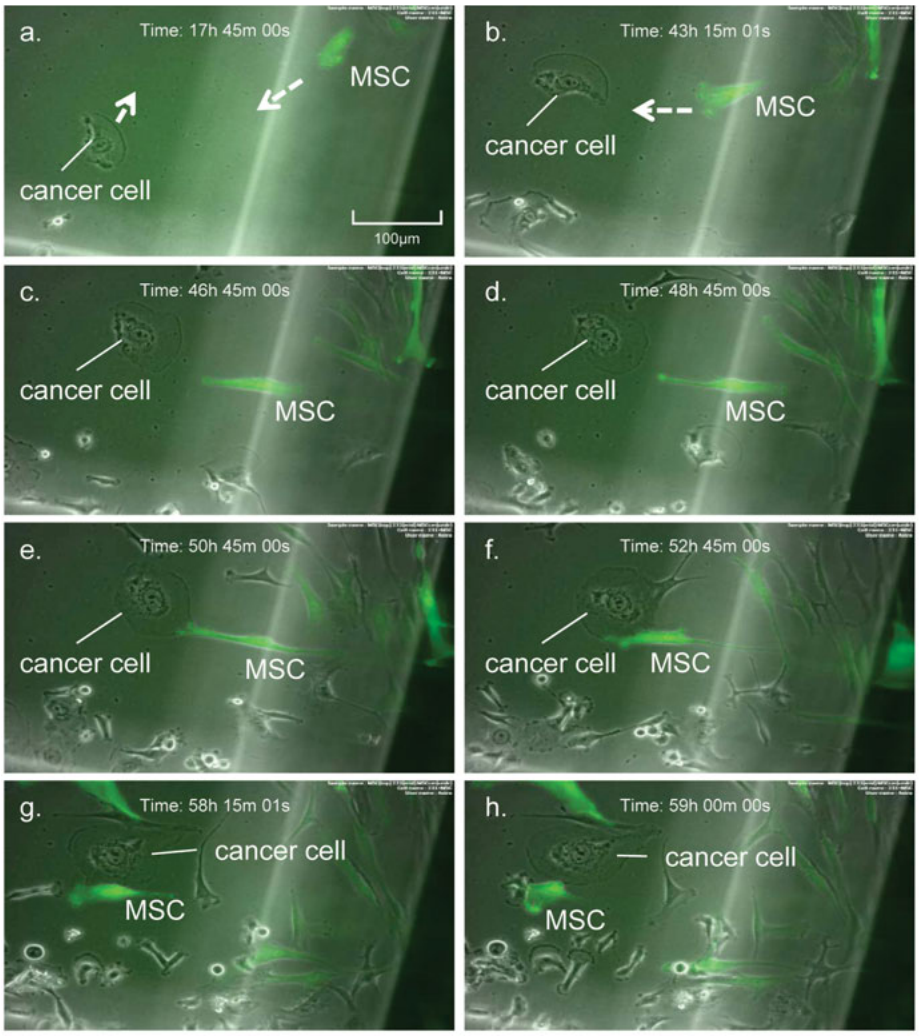

Fig. 18.5: Mesenchymal stem cells (MSCs) migrate towards cancer cells and communicate *via* direct contact. Shown here are still images of a time-lapse video recording of an under-agar assay in which breast cancer cells (MDA-MB-231) attract MSCs (green). As with most cells, MSCs will directly probe the cancer cells possibly sending and picking up signaling exosomes, *etc*.

may deliver their own exosomal packets to other cells. This may be one vital way in which MSCs are able to play their ambulatory role. For example, in the kidney, MSCs will protect against acute tubular injury *via* the horizontal transfer of mRNA to tubular epithelial cells by exosomal delivery, which confers to the tubular epithelial cells apoptotic resistance and functional recovery [41]. Similarly, exosomes secreted

by MSCs have been shown to reduce infarct size in a mouse model of myocardial ischemia/reperfusion injury [42].

Isolated exosomes from bone marrow-derived MSCs have also been shown to favor tumor growth and angiogenesis [43]. Certainly unknown factors in the tumor stimulate the release of such exosomes for delivery of needed factors for the continued dominant growth and survival of cancer cells. In a xenograft model of breast cancer, it was recently revealed that cancer-associated fibroblasts (CAF) secrete exosomes which potently stimulated protrusive activity and motility of breast cancer cells (MDA-MB-231) and that this activity is dependent on the exosomal protein CD81 [44]. But what if scientists were able to manipulate this tumor environmental exosomal release such that rather than the pro-survival growth factors, mRNA or other molecules, the exosomes delivered by MSCs instead contained potent anti-survival, anti-invasive, or anti-metastatic molecules, perhaps a silencing of key molecules such as exosomal CD81. Such manipulation of MSCs-delivered exosomes as drug-delivery vehicles is already being explored for therapeutic treatment of other diseases.

Current application of exosomes is hampered by drug loading strategies, which are currently being optimized as our understanding and characterization of exosomes increases. Thus, these exosomes may soon reach their enormous therapeutic potential. The two different strategies being developed involve *in vivo* loading during the intracellular biogenesis of the exosomes, or *in vitro* loading of isolated, purified exosomes [45]. Exosomes are an advantageous alternative to currently used liposomes since, like liposomes they are able to deliver their contents across the cytoplasmic membrane and provide a barrier to premature elimination. But unlike liposomes, exosomes are naturally occurring, less toxic, and better tolerated. They also have intrinsic homing ability conferred by the presence of specific ligands on their surfaces that interact with complimentary receptors on their targeted cell recipients. These membrane ligands are amenable to manipulation *in vitro* as are their contents, thus allowing the loading of therapeutic agents for tissue-specific homing. In the case of cancer, tumor-specific homing is enhanced by the exosomes being delivered by MSCs, which will naturally home to the tumor bed including metastatic ones that may not otherwise be detected. Furthermore, exosomes secreted by MSCs have the added advantage for this use in that they are immunologically inert.

18.5 MSCs as a gene product-delivering vehicle

18.5.1 Genetically modified MSCs for therapeutic delivery

Various different vectors have been used in studies to deliver gene silencing (*e.g.* siRNA) and gene-directed enzyme/prodrug therapies. Most often viral vectors such as adenoviruses [46], adeno-associated virus or lentiviruses have and are being utilized in clinical trials. But these viral vectors have been unsuccessful in transducing

tumors with effective levels of therapeutic genes, due to various reasons including the inability of the vector to penetrate the tumor mass or to reach distant metastasizing cancer cells. By taking advantage of the intrinsic migratory and communicative properties of MSCs to cancer cells this major obstacle of effectively delivering therapeutic genes can be overcome. MSCs are most often isolated from bone marrow, although other sources such as umbilical cord and placenta are quickly becoming viable options, expanded and genetically modified *in vitro*. In fact, the accessibility to genetic modification and expansion capability make MSCs ideal vehicles for tumor-targeted gene therapies, prodrugs, and cytokines or chemokines. For example, rather than patients having to take lengthy broad-acting chemotherapy infusions, in the future they might be administered a single set of engineered MSCs expressing various anti-angiogenic molecules for tumor suppression. Various methods to introduce these genes into MSCs have been successfully used, including viral transduction using adenovirus (especially oncolytic adenovirus, described below), measles virus, retroviruses, lentiviruses, or by OriP/Epstein–Barr virus nuclear antigen (EBNA)-based episomal plasmids, or recently transposon-based gene vectors. Studeny *et al.* performed one of the first applications of MSCs as a delivery vehicle in which they transduced MSCs with adenoviral vectors to introduce expression of interferon-β (IFN-β) [47]. The transduced MSCs were injected intravenously to mice with established melanoma xenografts and resulted in an inhibition of tumor growth as well as prolonged survival. Since this study, several other genes, such as TRAIL or cytokines, have been transfected or transduced into MSCs of different sources to treat a variety of cancer types.

18.5.2 Potential for MSCs-delivered anti-angiogenic therapies

Despite potential inadequacies in targeting angiogenesis as a single process in tumor growth and spread, halting tumor angiogenesis is a critical approach in ceasing cancer progression. As described earlier, tumors achieve angiogenesis in part by acting as a nonhealing wound including the recruitment of inflammatory cells that create a sort of smoldering inflammation that may promote malignancies. Various inflammatory factors play important roles to either promote or inhibit tumor angiogenesis, and many other aspects of tumor growth, cancer progression and metastasis [48]. It therefore follows that engineered MSCs targeting inflammation may be an excellent therapeutic option that can halt several processes at once. For example, proinflammatory interleukin-12 (IL-12) [49] has been demonstrated to have strong antitumor and anti-angiogenic effects [50]. However, systemic administration of IL-12 is also associated with severe toxicity [51]. To solve this problem, MSCs presents an ideal vehicle for delivery of the cytokine to the tumor site. In the study by Ryu *et al.*, glioma-targeting MSCs derived from umbilical cord blood were engineered to secrete a modified form of IL-12 having a higher T-cell helper 1 (Th1) and antitumor immunity

potency. At seven days post-treatment, significantly decreased tumor blood vessels as well as increased apoptotic cells were demonstrated in mice bearing intracranial gliomas xenograft compared to those treated with PBS- or unengineered MSCs [49]. The MSCs-delivered anti-angiogenic therapeutics may be enhanced with additional genes such as semaphorin 3A, under tumor-triggered expressional control, which help relieve the hypoxic pressure that may trigger epithelial-mesenchymal transition (EMT) and other metastatic events [52].

18.5.3 MSCs-mediated tumor-homing of oncolytic adenovirus enhances tumor therapy

Ideally, an oncolytic virus would selectively target malignant cells, infecting them and self-amplify by replicating within cancer cells, ultimately killing them. Oncolytic adenovirus has been shown to be effective in suppressing tumor growth, even completely eradicating colon xenografts after intratumoral injection [53]. Nevertheless, as previously discussed, intratumoral injection would not be suitable for many tumor types and distant or multiple metastatic sites. Furthermore, the distribution of adenoviral infection within the tumor would not be even. Intravenous adenoviral administration could lead to high levels of liver infection and toxicity, and cause a strong immune response to eliminate the virus. Yet another setback is that adenovirus does not have tumor-specific tropism. Despite this, oncolytic viruses continue to be pursued by some companies taking them into clinical trials [54]. Therefore, these oncolytic adenoviruses need a cancer-preferential carrier to reach tumor sites. Clearly, MSCs with their innate tumor-homing feature would be an ideal carrier for recombinant oncolytic adenoviral vector to reach the primary tumor and any distant metastatic sites.

Yong *et al.* employed human bone morrow-derived MSCs labeled with green fluorescent protein and carrying Δ24-RGD (hMSCs-Δ24) into the carotid artery of mice harboring orthotopic U87MG or U251-V121 xenografts. They found that there was an increase in accumulation of MSCs in the xenografts, and these MSCs released adenoviral vector infecting brain tumor cells. The tumor growth was suppressed, and some mice completely eradicated the tumor, and extended their survival from 42.4 days to 75.5 days, as compared to controls. This study proved the efficacy of MSCs-mediated recombinant oncolytic adenoviral delivery to the tumor site with improved eradication and animal survival [55]. A recent study utilized a mesenchymal stromal cell subpopulation (MO-MSCs), which displayed enhanced adhesiveness towards melanoma tumor xenografts. When these cells were loaded with oncolytic adenovirus and systemically administrated into mice harboring melanoma, the MO-MSCs suppressed tumor growth, and overcame the natural resistance of the tumor to the oncolytic adenovirus [56]. While not all studies have proved that MSCs exhibit tumor tropism, there is a definite improvement in the inhibition of tumor growth [57]. For example,

for hepatocellular carcinoma, active recruitment of MSCs into its xenografts has been confirmed by [124I]-PET imaging and immunohistochemistry [58]. Thus, it appears that MSCs tumor tropism depends on tumor type and the recognition of tumor surface markers by MSCs.

18.5.4 Delivery of TRAIL by genetically modified MSCs to induce apoptosis

In addition to being a carrier of oncolytic virus, genetically modified MSCs may work as a local factory producing therapeutic agents adjacent to tumor cells, and exert anti-tumoral effects *via* suppressing anti-angiogenesis, inducing apoptosis or intervening metastasis [59]. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), also known as Apo Ligand 2 (ApoL2) is a pro-apoptotic protein that binds to cancer cells expressing death receptors 1 and 2. This protein is being extensively studied for its anticancer properties [53] and has been in clinical trials in late 2012 (www.clinicaltrials.gov). Despite these studies, the rapid clearance of TRAIL remains a challenge. TRAIL in a soluble form has a half-life of approximately 30 minutes [60], which is increased to approximately 15 hours when it is fused with carrier proteins such as human serum albumin. Moreover, the delivery of adenovirus encoding the TRAIL gene by MSCs was less immunogenic, and inhibited the growth of lung cancer xenografts in mice [61]. However, TRAIL may also have toxic effects on normal tissues such as the brain and liver. For these reasons, Reagan *et al.* aimed to introduce site-specific TRAIL expression by MSCs [45]. They designed an implant delivery system, and used a doxycycline-inducible promoter to control the expression of the TRAIL gene in the engineered MSCs [38, 46]. Tet-on and Tet-off systems may modulate gene expression of a cytotoxic protein in a controlled manner, and would be particularly useful in this case [62]. In other studies, the initiation of a particular gene expression by the tumor environment has been used to drive tumor-specific therapeutic expression. The promoter of the gene being turned on by the tumor environment is used to drive the expression of the therapeutic gene.

18.5.5 Tumor-specific promoter-driving thymidine kinase (TK) expression for prodrug conversion

Karnoub *et al.* demonstrated in a xenograft model that breast cancer cells actively recruit MSCs to the tumor environment. Once the MSCs are in relatively close proximity, the cancer cells will induce a potent upregulation of CCL5/RANTES gene expression [63]. CCL5/RANTES is a potent molecule stimulating migration and other metastatic mechanisms (see Fig. 18.6 and Section 18.9.3 for details). Bruns *et al.* took advantage of this property and utilized the CCL5/RANTES gene promoter to drive the expression of the suicide transgene HSV-TK in engineered MSCs with the use of ganciclovir,

Table 18.1: Selected summary of experimental studies of MSCs engineered for anticancer therapies.

Ref.	Source of MSCs	Type of MSCs-mediated therapeutic	Cancer Type	Observed Effects				
				Growth Inhibition	Reduced Tumor Size	Inhibition of Metastasis	Prolonged Survival	Other
Studeny [47]	BM	MSCs-transfected to express IFN-β	Melanoma	Yes	No	Suppression of pulmonary metastasis	Yes	
Loebinger [93]	BM	Tet promoter-driven MSCs-expression of TRAIL	Lung, breast, cervical	Yes	Yes	Clearance of pulmonary metastasis	Not reported	Clearance of micro-metastasis Some complete eradication
You [82]	BM	MSCs-transfected cytosine deaminase plus 5-fluorouracil prodrug treatment	Gastric	Yes	Yes		Not measured	Minimized side effects of 5-fluorouracil
Zischek [64]	BM	CCL5 promoter-driven HSV-TK suicide gene expression (with ganciclovir treatment)	Pancreatic	Yes	Yes	Reduced metastasis to peritoneum, spleen and liver	Not reported	
Conrad [66]	BM	Tie-2 promoter-driven HSV-TK suicide gene expression (with ganciclovir treatment)	Pancreatic, breast	Yes	Yes		Yes	
Reagan [45]	BM	Dox-induced TRAIL-expressing MSCs	Breast	Yes	Yes	Decreased bone, lung and liver metastasis	Not reported	
Zolochevska [94]	Adipose	MSCs-delivered pigment epithelial-derived factor (PEDF)	Prostate	Yes	Yes		Not reported	Prevented tumor establishment
Yong [55]	BM	MSCs-delivered oncolytic adenovirus	Brain	Yes	Yes		Yes	Some complete eradication

in a syngeneic model of pancreatic cancer [64]. Ganciclovir (GCV), a strong antiviral medication commonly used to treat and prevent cytomegalovirus (CMV) infection, is phosphorylated by HSV-TK resulting in an active deoxynucleotide analogue. As cell division occurs and DNA is synthesized, the incorporation of the ganciclovir/HSV-TK generated nucleotide terminates strand synthesis and arrests cell division [65]. Combining the selective migration of the engineered MSCs to tumors with the efficacy of the GCV/HSV-TK "suicide gene" system allows for highly selective tumor targeting using MSCs.

Another HSV-TK/GCV suicide gene therapy method being explored uses Tie-2 gene as a target, which is upregulated in tumor neoangiogenesis and is responsible for stimulating angiopoietin receptor tyrosine kinase activity (angiopoietin-TIE system), important in the tumor angiogenic switch. This system represents an alternative to VEGF-A in targeting tumor angiogenesis with several studies showing promising anticancer activity in early clinical trials. One study involving engineered MSCs demonstrated that in the MSCs differentiating towards tumor endothelial-associated cells, Tie-2 is upregulated, thus activating the HSV-TK/GCV suicide gene system. This resulted in reducing tumor volume in mice without the need for myeloablative therapy [66]. The use of Tie-2 promoter/enhancer elements to drive therapeutic gene expression in MSCs allows for the selective expression of these genes only after the MSCs have homed to the tumor bed and they have been stimulated to suppress tumor angiogenesis, and is therefore a very promising therapeutic use of MSCs for tumor targeting.

Table 18.1 summarizes several examples of MSCs-mediated delivery of therapeutics to xenografts in animal models of various tumor types. Most of the delivery methods used were *via* intratumoral injection, although genetically engineered MSCs have been demonstrated to exhibit tumor-homing property in these studies. However, it should be noted that intravenous administration of MSCs for lung cancer or metastatic sites yielded the first pass deposition of MSCs in pulmonary circulation [59]. Therefore, routes of MSCs administration remain to be carefully investigated in the translation of promising MSCs-mediated delivery of therapeutics for targeting tumor therapy.

18.6 Methods of therapeutic MSCs administration

While direct injection of therapeutically engineered MSCs may appear to be the best method, it is also obviously problematic for tumors located in tissue areas difficult to reach. Other less obvious reasons may also suggest that this may not be an advantageous approach. Over a decade ago, Ram *et al.* attempted to directly inject therapeutically engineered MSCs to the tumor. They injected MSCs expressing murine herpes simplex virus-thymidine kinase (HSV-TK) transgene intratumorally to patients with recurrent malignant brain tumors [67]. Unfortunately, the results were disappointing. Since then knowledge acquired in the field suggests that normal physical processes

associated with MSCs recruitment from the circulation may impart some imprinting through their passage which may be important to their physiology once within the tumor environment [68]. Most studies involving *in vivo* application of MSCs-engineered anticancer therapies now utilize intravenous injections to introduce the MSCs. This carries the advantages of being less invasive and allowing for intrinsic mechanisms of MSCs to guide them into tumor niches that may not necessarily be detectable or identified otherwise. The question remains whether the majority of injected MSCs will reach the tumor sites. In another words, what is the efficiency of MSCs-mediated delivery of drug, gene, virus or siRNA to the tumor surrounding or tumor parenchyma?

A preclinical study explored a delivery approach involving the implantation of silk scaffolding that provides a niche environment within which MSCs may be seeded [45]. Advantages for using silk scaffolding rather than a decellularized matrix is that silk is already used extensively in medical practice, and may be used in various forms with different sizing, mechanical strength, porosity, and may also be modified for degradation time from weeks to years [69]. Three different methods of administration were compared – co-injection of the MSCs expressing TRAIL under doxycycline control with breast cancer cells, tail vein injection of the MSCs, and implantation of the MSCs seeded on the silk scaffold. The study demonstrated that breast cancer cells recruited the engineered MSCs from the implanted silk scaffold to the tumor site [45, 70] and resulted in significant reductions in tumor growth. The study concluded that tail vein injection of the therapeutic MSCs resulted in decreased bone, lung and liver metastases, as did implantation of the therapeutic TRAIL-expressing MSCs on the biocompatible silk implant, with the exception of liver metastasis [45]. It appears therefore that such implants may be a valuable approach translatable for long-term treatment to inhibit tumor growth and help diminish at least some forms of metastasis.

18.7 The advantage of MSCs being immunoprivileged

Mesenchymal stem cells have a unique property of being immunoprivileged. This is an important and highly advantageous characteristic for the utilization of MSCs for allogeneic delivery of therapeutic genes and other molecules. This immunoprivileged nature is due to several different mechanisms acting on various different types of immune cells all in a coordinated fashion. MSCs are resistant to natural killer (NK) cell cytolysis, and inhibit NK cell proliferation and the generation of dendritic cells and macrophages [71]. MSCs inhibit proliferation and induce apoptosis of activated T cells [72], while also altering their migratory properties along with that of dendritic cells [73]. Another important component contributing to the immunoprivileged nature of MSCs is that these cells lack expression of MHC class II, as well as CD40, CD80, and CD86 costimulatory molecules. Importantly, the immunoprivileged nature of MSCs allows for the use and delivery of normal donor (allogeneic) MSCs without immuno-

modulation or subsequent immunosuppressive therapies to a wide patient population, made possible also by the highly proliferative nature of low passage MSCs *in vitro*. Thus, large batches of qualified, therapeutic MSCs may be prepared in good manufacturing practice (GMP) facilities and stored for future use in numerous cohorts of patients. This has already been demonstrated *in vitro*, *in vivo*, as well as in Phase I through Phase III clinical trials for the treatment of autoimmune diseases and in graft-versus-host disease (GvHD) for patients receiving hematopoietic cell transplantations [74]. Non-genetically-modified MSCs have been approved as drugs for GvHD in Canada, New Zealand, and Korea due to strong safety profiles. The use of MSCs as a carrier for antitumor therapeutics is an excellent example of potential personalized medicine that can be expanded to reach a large breadth of patients, and will be very valuable to oncology therapies.

18.8 Sources of acquiring MSCs for tumor therapy

The bone marrow is a primary source of nonhematopoietic and highly proliferative MSCs, holding differentiation ability. Standard isolation of MSCs from the mononuclear fraction of bone marrow aspirates involves the depletion of $CD45^+$ cells and adherence to plastic tissue culture dishes. Fibroblastic cells and macrophages are separated from MSCs in that they will adhere more strongly such that a standard enzymatic lift will leave these strongly adherent cells behind, releasing MSCs. Qualification of the MSCs populations to ensure that no macrophage or hematopoietic cell contamination remains in cultures must be done prior to use. Bone marrow MSCs are probably the most widely characterized and thus most widely used sources of MSCs in part due to their ready availability. However, MSCs can be isolated from adipose tissue, liver, lung, placenta, and even teeth [75]. Adipose tissue as a source of therapeutic MSCs is becoming more popular. Indeed, plastic-adherent adipose-derived stem cells appear not to solicit a T-cell response; and late-passage cells act to inhibit reactions of mixed populations of lymphocytes [76]. The umbilical cord can also be a rich resource for MSCs. MSCs are isolated from umbilical cord tissue that has been washed of any surrounding blood and striped of the umbilical cord veins. What remains is also known as Wharton's jelly and MSCs can readily be isolated from cultured explants. MSCs have also been isolated from amnion and subamniotic tissues [77, 78], as well as perivascular tissues surrounding the large umbilical cord veins.

Despite their origins from various sources, MSCs have a general fusiform shape and are able to actively move around. Their capacity for differentiation to adipose, bone, and cartilage lineages, as well as to pericytes and endothelial-associated cells, is part of the gold standard above the minimum criteria for characterizing MSCs [23]. Minimum criteria may include characterization by cell-surface markers which requires the use of a panel of antigens giving the signature $CD105^+$, $CD73^+$, $CD90^{hi}$, $CD14^-$, $CD34^-$, $CD19^-$, HLA-DR^-, $CD45^-$ [79]. As described in a previous section, differ-

ences in the antitumor actions of nonmanipulated MSCs are observed dependent on the tumor type but also on the source of MSCs. Therefore, further characterization of the differences between MSCs from various sources is essential for data interpretation and consistence.

18.9 Remaining challenges for the use of MSCs to deliver therapeutics

18.9.1 The immunoprivileged nature of MSCs

While the immunoprivileged nature of MSCs is clearly advantageous in the development and application of MSCs as a wide-ranging therapeutic biocarrier, some fear that their immunosuppressive properties may pose to be problematic in that they may further free cancer cells from immune surveillance and attack. In other words, therapeutic allogeneic MSCs, while clearly advantageous in being able to treat large numbers of patients, may also be immunosuppressive. Nonetheless, most aggressive tumors have already undergone immune escape in their early establishment, which allows them to continue to grow and expand. Thus, while important to keep in mind, the immunoprivileged nature of MSCs may turn out to not be a major cause for concern.

18.9.2 Varying responses to MSCs depending on cancer type, injection site, etc.

In the translational application of therapeutically engineered MSCs to various cancer types, caution should be taken as not all cancers may respond in a positive manner. While some researchers report MSCs aiding in tumor growth, for example like other stromal cells which may undergo autophagy to help feed the cancer cells [11], others have documented a reduction in tumor growth by MSCs. In fact, MSCs may participate in a balance of the two, and the discrepancies in published studies may stem from the timing of experimental MSCs administration [80]. A key example is given by gastrointestinal cancers, in which some conflicting results from studies with therapeutic MSCs have been observed. For example, tumor progression was observed in an esophageal cancer when MSCs were subcutaneously injected together with the cancer cells to nude mice, after the MSCs were shown to inhibit proliferation and invasion *in vitro* [81]. Nonetheless, profound tumor growth inhibition in a gastric cancer mouse model was observed when therapeutic MSCs engineered to express the suicide gene cytosine deaminase were administered in combination with the prodrug 5-fluorouracil (5-FU) [82]. Furthermore, Wang *et al.* showed that bone marrow-derived MSCs reduced tumor progression in a *Helicobacter felis*-induced gastric dysplasia model [83]. Taken together, these three studies implicate that (1) *in vitro* results may not always translate

to the *in vivo* model especially in oncogenic studies in which full understanding of the complicated milieu of signaling processes is still being elucidated; (2) nonengineered MSCs may have a beneficial anticancer progression effect depending on the type of cancer; and finally (3) therapeutically engineering the MSCs may have a greater antitumoral effect than unengineered MSCs.

18.9.3 Changes in MSCs induced by cancer cells within the tumor microenvironment

It is now clear that cancer cells stimulate or repress the expression of various genes in MSCs and other cells. For example, the discovery that CCL5, also known as RANTES (regulated upon activation, normal T-cell expressed and secreted) is specifically upregulated and secreted by MSCs in the presence of breast cancer cells was made by Karnoub *et al*, who described a role of CCL5 in the metastasis of breast cancer [63] (Fig. 18.6). The precise mechanism through which cancer cells initially stimulate the secretion of CCL5 from the MSCs is not fully understood. Recently, Mi *et al.* provided

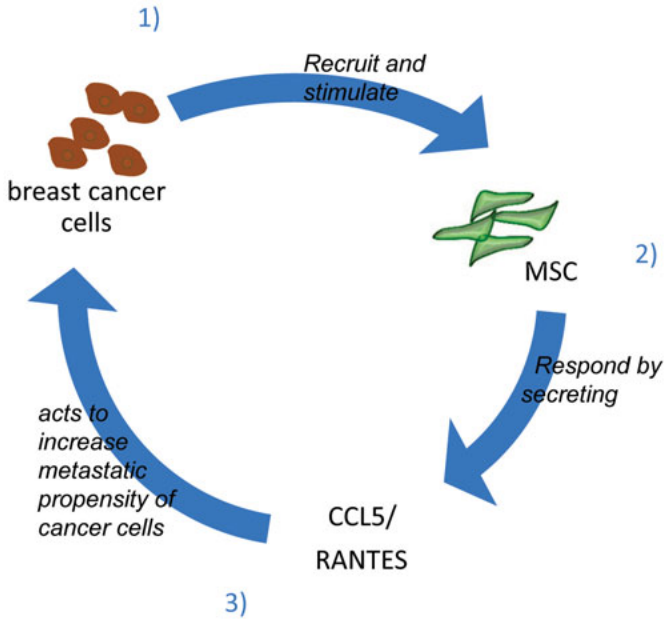

Fig. 18.6: Mesenchymal stem cells (MSCs) are recruited by breast cancer cells (MDA-MB-231). MSCs have been demonstrated to be stimulated by breast cancer cells to dramatically increase the expression and secretion of CCL5/RANTES with the cytokine subsequently acting in a paracrine manner upon the cancer cells to increase migration and other metastatic mechanisms.

initial evidence that tumor-derived osteopontin (OPN) may induce the production and secretion of CCL5 [84]. Osteopontin is highly expressed in tumors cells and, as illustrated earlier in Figures 18.4 and 18.6, may act on MSCs to cause the upregulation of CCL5 expression and secretion from MSCs. The chemokine CCL5 acts back on the cancer cells in a complimentary paracrine fashion as a chemoattractant interacting with receptors to increase mobility toward the MSCs [84, 85].

Increased HIF-1α expression and activation in cancer cells will result in increased expression of the receptor for CCL5, namely CCR5 [86]. However, it is very interesting that secreted CCL5 will additionally interact with CD44 [87, 88]. CCL5 binds with CD44 on the cancer cells and signals to enhance their mobility, invasive properties, and proliferation, resulting in an enrichment of tumor initiating cancer stem cells [85] likely through a CD44-intracytoplasmic domain response element. The CD44 intracytoplasmic domain (CD44-ICD) cleaves apart from the transmembrane protein, translocating itself within the nucleus [89]. Of note, cancer cells do not require a hypoxic environment to activate expression of HIF-1α genes. Here, CD44 is capable of activating HIF-1α responsive genes independent of a hypoxic environment, by binding to novel DNA consensus sequences that constitute a CD44-ICD response element in the promoter region of these genes. The expression of these genes results in an increase in cancer cell motility, increased cell survival, and tendency to undergo differentiation [89].

Thus, in this cross-talk, cancer cells may recruit MSCs *via* IL-6 and perhaps other pathways, stimulate them in part by secreting OPN (and possibly other molecules) thus causing MSCs to secrete CCL5 which may bind to CCR5 on cancer cells and/or CD44 on cancer stem cells. The CD44-ICD is cleaved from the membrane protein, traverses the cytosol to the nucleus binding to response elements in the promoter regions of HIF-1α responsive genes, and turns on or increases the expression of signaling pathways that aid cancer cell mobilization, proliferation, invasion, and ultimately metastasis. For these reasons, scientists believe that suppressing the communication between MSCs and cancer cells could have potential as a therapeutic target (*e.g.* zoledronic acid suppression of bone marrow MSCs decreases breast cancer cell migration) [90].

Importantly, however, the therapeutic potential is enormous if, instead of secreting tumor-promoting ligands when stimulated by cancer cells, MSCs can be engineered to secrete a deadly pharmaceutical molecule or gene product that will induce apoptosis in cancer cells, stop tumor growth, or halt metastatic spread. Through understanding the effects of the tumor environment on MSCs and how MSCs are attracted there, scientists may be able to best engineer "Mesenkillers" in fighting against cancers. Endless targeting possibilities arise if these properties of MSCs to migrate to and to be stimulated by the tumor microenvironment can be taken advantage of and utilized, such as with use of tumor-activated promoters.

18.10 Summary and prospective

Currently, radiation and chemotherapy are the standard of care for many types of cancers that are not suitable for surgical resection. A common side effect of such chemotherapy is suppression of the hematopoietic function of bone morrow along with effects on other systems. An early clinical trial using MSCs helped improve the hematopoietic recovery of patients having undergone chemotherapeutic treatment. Participants were breast cancer patients who had received myeloablative therapy, and the results of the trial demonstrate that MSCs improved hematopoietic recovery (without enhancing relapse) [91]. This was a clear demonstration of the enormous therapeutic potential of MSCs, in addition to genetically engineered MSCs for the delivery of therapeutics. The most promising prospects include MSCs-mediated oncolytic adenovirus for improved selective killing of tumor cells, prodrug delivery to the tumor site, thymidine kinase or TRAIL expression to induce apoptosis in a controllable fashion, generation of silencing molecules (*e.g.* antibody, siRNAs) at tumor sites for direct antiangiogenesis or specific inhibition of molecules critical for tumor growth, progression and metastatic pathways, such as Wnt signaling, EGFR signaling, and so on [92]. MSCs-mediated adenoviral delivery has been shown to not only reduce systemic toxicity of the recombinant adenovirus, but also enhances its cytotoxicity to tumor cells. The ability to track engineered MSCs, for example using biocompatible magnetic nanoparticles, will be a valuable tool to carefully evaluate the tumor-homing property and the longevity of MSCs after intravenous administration. Such tracking would be a highly advantageous and noninvasive modality to verify the therapeutic use of MSCs while confirming the selective delivery of therapeutics to tumor site. Despite this great potential, more research is needed to determine the tumor-suppressing benefits against possible tumor-promoting effects, the extent and the significance of immune suppression after MSCs administration, and the safety profiles of the therapeutics and the MSCs carrier. The translation of this "double-edged sword" yet potentially effective cell therapy approach, aimed at improving the current outcome of cancer treatments, to clinical application will take a reasonable time period. Nonetheless, this appears not too far in the future as the drug regulatory bodies of some countries, including Canada and New Zealand, have already approved the use of MSCs as a biologic therapy.

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