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Mesenchymal stem/stromal cells: Developmental origin, tumorigenesis and translational cancer therapeutics

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ABSTRACT

While a large and growing body of research has demonstrated that mesenchymal stem/stromal cells (MSCs) play a dual role in tumor growth and inhibition, studies exploring the capability of MSCs to contribute to tumorigenesis are rare. MSCs are key players during tumorigenesis and cancer development, evident in their faculty to increase cancer stem cells (CSCs) population, to generate the precursors of certain forms of cancer (*e.g.* sarcoma), and to induce epithelial-mesenchymal transition to create the CSC-like state. Indeed, the origin and localization of the native MSCs in their original tissues are not known. MSCs are identified in the primary tumor sites and the fetal and extraembryonic tissues. Acknowledging the developmental origin of MSCs and tissue-resident native MSCs is essential for better understanding of MSC contributions to the cellular origin of cancer. This review stresses that the plasticity of MSCs can therefore instigate further risk in select therapeutic strategies for some patients with certain forms of cancer. Towards this end, to explore the safe and effective MSC-based anti-cancer therapies requires a strong understanding of the cellular and molecular mechanisms of MSC action, ultimately guiding new strategies for delivering treatment. While clinical trial efforts using MSC products are currently underway, this review also provides new insights on the underlying mechanisms of MSCs to tumorigenesis and focuses on the approaches to develop MSC-based anti-cancer therapeutic applications.

Introduction

Human mesenchymal stem/stromal cells (MSCs) have drawn attention as a means to cellular therapy in regenerative medicine due to their properties of immunomodulation, self-renewal and tissue regeneration. MSCs can be harvested from multiple sources, including adult bone marrow, adipose tissue, peripheral blood and various neonatal birth-associated tissues and they can also be induced *in vitro* to differentiate into osteoblasts, chondrocytes, adipocytes, and other cell types [1]. There is extensive and active clinical activity in the interaction of MSCs with cancer. To date, clinical investigations utilizing MSCs as delivery vehicles for tumor-targeted gene therapy are being explored due to their unique therapeutic properties for genetic modification *in vitro*.

Human MSCs are isolated at a very low frequency from bone marrow at approximately 0.001% of the total nucleated cell population [2]. *Ex vivo* expansion of human MSCs is therefore necessitated to obtain sufficient numbers prior to regenerative medical applications. Different laboratories employ disparate methodologies to isolate and expand MSCs, thus eliciting numerous inconsistencies in their cellular characterization. To address this issue, the International Society for Cellular Therapy (ISCT) proposes minimal criteria defining *in vitro*-expanded MSCs, including adherence to plastic, specific surface antigen expression and multi-differentiation potential [3]. MSCs derived from different tissues exhibit varied phenotypic and functional behavior and should not be given the same name. There is considerable controversy surrounding the term "stem cells" in MSC nomenclature. The ISCT's MSC committee recommends the functional definition of mesenchymal stem *versus* stromal cells to further clarify the nomenclature of mesenchymal stromal cells (MSCs) [4]. The MSC committee continues to support the use of the acronym "MSCs", but recommends this be: (i) supplemented by tissue-source origin of the cells; (ii) intended as MSCs unless rigorous evidence for stemness exists that can be supported by both *in vitro* and *in vivo* data; and (iii) associated with robust matrix of functional assays to demonstrate MSC properties [4].

The contributory role of MSCs in tumor progression and metastasis is a subject of active debate. MSCs possess the properties of both tumor

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suppression and promotion through a variety of mechanisms [5,6]. For example, MSCs can play a dual role of inhibiting tumor angiogenesis [7] or promoting tumor vascularization [8]. To date, studies performed on the capability of MSC contributions to tumorigenesis are scarce. In this review, we distill our discussion on how MSCs contribute to the cellular origin of cancer, emphasizing a few important aspects of the field: the developmental origin of MSCs and the localization of MSCs in fetal and extraembryonic tissues; the most likely progenitors of tumorigenic cells in sarcoma; and maintaining cancer stemness by MSCs. In closing arguments, we discuss the combinational strategies to stress therapeutic safety and efficiency for exploring MSC-based anti-cancer therapies. While the specific role of MSCs in tumorigenesis is far from being completely clarified, it is critical to appreciate the mechanisms of action to guide the development of MSC-based cancer therapeutics.

MSCs in primary tumor sites and their developmental origin

Given the diversity in tissue-specific properties of MSCs, the tissue of origin of MSCs should necessarily be provided, such as bone marrow-derived MSCs (BM-MSCs). The present review's focus on MSC contributions to the cellular origin of cancer should not detract from acknowledging the developmental origin of MSCs and the localization of MSCs in fetal and extraembryonic tissues.

Primary tumor-resident MSCs

Interestingly, previous works revealed that MSCs are invariably present in primary tumors and in the primary sarcoma. We have reviewed up-to-date knowledge available on resident MSCs/mesenchymal progenitor cells in the established tumors *in situ* that are also found in different primary tumor types, including human breast cancer [9], hepatocellular carcinoma [10] and osteosarcoma [11]. We sought to determine whether these MSCs at sites of primary cancer *in situ* are from endogenous local sources during fetal development or from an influx of distant MSCs reservoirs, but the lack of a well-accepted standard for the identification of endogenous MSCs maintains uncertainty regarding their source. MSCs act as a precursor of the certain tumorigenic cells and this concept has initially emerged from MSC development studies. Our primary interest is targeted to the developmental origin and localization of MSCs in fetal and extraembryonic tissues.

Origin of MSCs during early embryonic development

Human MSCs are identified and characterized in fetal liver as early as 7 weeks' gestation [12]. MSCs are also detected in human first-trimester fetal blood at the 7th week and bone marrow from the 10th week [13]. Going further back, MSCs are detected in aorta-gonad-mesonephros, yolk sac, and urogenital sinus (developed into the prostate) as early as the day 25 of developing human embryos [14], prior to their emergence in fetal blood, liver and bone marrow.

The development of MSCs is poorly understood. The mammal (principally the mouse) embryonic stem cells (ESCs), the most pluripotent stem cells (PSCs), are derived from the inner cell mass of a cyst [15,16], an early-stage preimplantation embryo. At the blastocyst stage, ESCs can proliferate and differentiate into the primitive ectoderm (a pluripotent population of cells) which then goes on to form the epiblast. Epiblast cells in the specific spatiotemporal stage in the ectoderm undergo the epithelial-mesenchymal transition (EMT) programs to generate MSCs and primordial germ cell progeny with activated cancer/testis (CT) antigens that later become inactivated as the result of cell differentiation [15,16]. These biological events that occur during *in vivo* embryonic development, including MSC proliferation, differentiation, migration, tissue localization and transition, need to be further emphasized. There is additional evidence that MSCs originate from perivascular cells, principally pericytes that are vascular mural cells, within multiple human organs including skeletal muscle, pancreas, adipose tissue and placenta [17], and emigrate into capillary walls in surrounding fibrous tissues during times of development [4]. The discovery that the human blood vessel walls harbor native MSCs may be one key to a better understanding of the developmental origin of MSCs.

CT antigens, potential biomarkers and immunotherapeutic targets for cancer, are frequently expressed in malignant tumors with limited expression in germ cells of the testis, fetal ovary, and placenta [18]. Importantly, multiple CT antigens have been confirmed to have unique expression profile in human cancer stem cells (CSCs), including lung CSC-like cells [19], multiple myeloma CSC-like cells [20], and acute myeloid leukemia CSCs [21]. Interestingly, several CT antigens such as *MAGE-A, NY-ESO*, and *SSX* are also expressed in human fetal and adult MSCs [12,22,23]. CT antigen expression might influence the cellular behavior of MSCs. The underlying mechanisms about how the activated and inactivated CT antigens are involved in MSCs development remain to be fully investigated.

Formation of MSC-like cells through EMT induction

EMT or the reverse process (MET) is a developmental cellular process and represents one important source of epithelial and mesenchymal cells. For example, EMT contributes to the genesis of epithelial stem-like cells from the large population of differentiated normal mammary epithelial cells [24]. Most interestingly, one previous report indicated that the kidney MSC-like cells are derived from the mature collecting duct epithelium that undergoes an EMT [25], albeit the underlying mechanism is poorly understood. These kidney MSC-like cells express the typical MSC immunophenotype and retain mesodermal differentiation potential [25]. Another previous report showed that EMT-derived cells from human breast epithelial cells exhibit functional and phenotypical similarity of MSCs [26]. These EMT-driven cells behaved similarly to MSCs to migrate toward MDA-MB-231 breast cancer cells.

Origin of MSCs through the endothelial-to-mesenchymal transition

There are other molecular approaches to identifying the multiple developmental origins of MSCs. MSCs have a distinct *in vivo* entity of the mesenchyme and the mesoderm is a major source of the mesenchymal precursors. Mesenchymoangioblast (MB) is identified as a mesoderm-derived precursor for mesenchymal and endothelial cells [27,28]. MB-derived primitive mesenchymal cells have the potential to differentiate into MSCs pericytes and smooth muscle cells. It is well known that the plasticity of epithelial and endothelial cells is critical for embryonic development. One study by Medici et al. shows that vascular endothelial cells can transform into mesenchymal stem-like cells that have the differentiation potential into multiple cell lineages through the endothelial-to-mesenchymal transition (EndMT) [29]. Expression of constitutively active activin-like kinase-2 (ALK2) in endothelial cells results in the transition of endothelium into mesenchyme. Similar results are obtained by ligand-specific induction of EndMT in an ALK2-dependent manner that examines ALK2 receptor phosphorylation through treatment with either transforming growth factor-β2 (TGF-β2) or bone morphogenetic protein-4 (BMP4) in endothelial cells. Takashima et al. have reported that MSCs arise from the neuroepithelium but not from the mesoderm in ESCs culture [30]. Using Cre-recombinase mediated lineage tracing, they carry out a persistent labeling experiment of $Sox1^{+}$ neuroepithelium as precursors of MSCs of neural crest origin.

As mentioned previously, native MSCs are known to reside in multiple fetal tissues. The identification of the developmental origin of MSCs and tissue-resident native MSCs is critical in understanding the cellular and molecular mechanisms that MSCs invariably present in the primary tumors in their native tissues. The current scope of available data has been condensed into a schematic diagram demonstrating the developmental origin and fetal tissue localization of MSCs in Fig. 1.

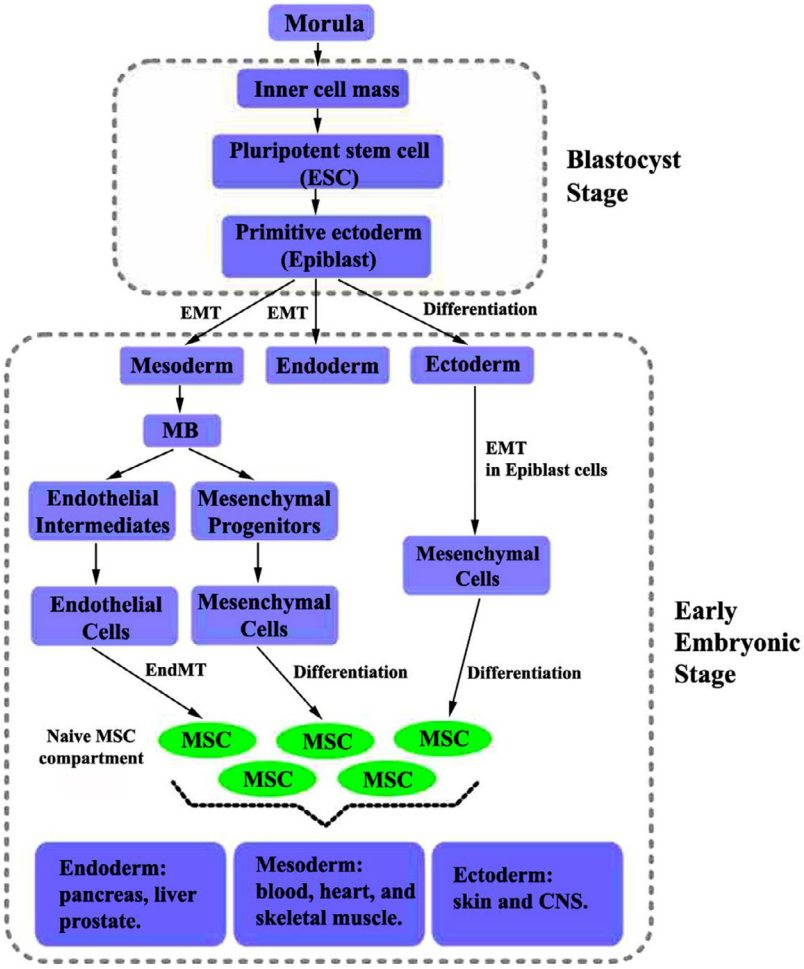

Fig. 1. The developmental origin and fetal tissue localization of the naive MSCs. During early mammal development, all cells are identical and undifferentiated before the formation of the blastula. The cells of the inner cell mass (ICM) begin to differentiate and divide rapidly during blastocyst stage. The ICM proliferates and differentiates to generate the epiblast and eventually to form the all three primary germ layers [15,16]. Meanwhile, the epiblast cells in ectoderm undergo the epithelial-mesenchymal transition (EMT) to generate MSCs [15,16]. Mesodermal cell-derived mesenchymoangioblast (MB) can differentiate into endothelial and mesenchymal cells and MB-derived endothelial cells can transform into mesenchymal stem-like cells through endothelial-to-mesenchymal transition (EndMT) [27–29]. MB-derived mesenchymal cells have potential to differentiate into MSCs.

MSCs: contributions to the cellular origin of cancer

While the numerous studies have demonstrated that MSCs may promote or suppress tumor progression through a number of well-documented mechanisms [5–8], cellular origins of a developing cancer associated with MSCs is poorly understood. MSCs are the most likely progenitors of certain tumorigenic cells or tumor-initiating cells (TICs) under certain circumstances, albeit the cell-of-origin of most cancers remains unknown. For example, bone marrow-derived cells (BM-MSCs) are frequently recruited to the sites of tissue injury in response to chronic Helicobacter infection and repopulate in mouse gastric mucosa, eventually resulting in metaplasia, dysplasia and cancer [31]. Evidence in human and animal studies suggests that neural stem cells act as the possible cell-of-origin for the central nervous system tumors [32,33]. It begs the question whether MSCs are also an alternate cellular origin of cancer.

Induction of emt by MSCs to generate CSCs

CSCs coexist as minority populations in a variety of solid tumors, but the origin of CSCs remains under debate. Under pathophysiological condition, EMT can also play a critical role in tumorigenesis and metastasis by inducing the conversion of non-CSC tumor cells into the CSC-like state [24,34,35]. For example, transcription factor Slug and Sox9 act cooperatively to induce breast cancer cells into the TIC state [34]. CSCs can also originate from normal tissue stem cells by EMT induction [34,36,37]. Morel et al. show that $CD44^{+}CD24^{-/low}$ cells possessing stem-like properties can be generated from $CD44^{low}CD24^{+}$ non-tumorigenic mammary epithelial cells following their transformation through an EMT process [36].

Importantly, some studies highlight the EMT induction of cancer cells by MSCs to increase the CSC populations [38–40]. For example, one previous study by Li et al. indicated that cancer-stimulated MSCs create a CSC niche via the release of cytokines and prostaglandin E2 [40]. In this study, Li et al. showed that MSCs can directly induce EMT in the cancer cells resulting in a concomitant entrance into the CSC-like state in response to MSC-derived heterotypic signals [40]. Another previous work by Martin et al. demonstrated significant up-regulation in specific EMT markers in breast cancer cell lines co-cultured with MSCs, when compared to co-culture with fibroblasts [41]. Additionally, mounting evidence indicates that CT antigens can also induce EMT programs and increase cancer stem-like cells [42,43]. Shang et al. have found that the CT45A1, one representative CT45 family member, promotes EMT and increases stemness in breast cancer MCF-7 cells, resulting in tumorigenesis and metastasis [43]. However, it is unclear whether CT antigen-expressing MSCs accelerate EMT induction to generate CSCs.

Mechanistically, the initiation and progression of EMT involve escalating rise in signaling molecule mediated complex cell-cell communication and the convergence of signaling pathways. Among these, TGF-β signaling has a predominant role in inducing EMT [35,44,45]. As an example, Mele and colleagues' studies in co-culturing of MSCs with human colorectal cancer cells found that MSCs strongly induce an EMT in human colorectal cancer cells through TGF-β signaling [44]. Silencing TGF-β expression in MSCs can revert the EMT-promoting effect, subsequently accompanied by the anti-proliferative and pro-apoptotic effect of MSCs on A549 lung cancer cells [45]. The EMT progression is regu-

lated through crosstalk of complex signaling pathways and the detailed molecular mechanisms of MSCs inducing EMT need to be further understood.

Maintenance of cancer stemness by MSCs

Tumor cells coordinate with various non-cancerous types of cell populations that reside in or are recruited into the tumor-associated stroma to create a complex tumor microenvironment (TME). MSCs are an integral cellular component of the TME and are now recognized as key players to tumor progression and metastasis. Tumors behave as unhealed wounds in the body [46]. The tumor process is highly related to inflammation and tumors themselves share many important properties with healing wounds [2,46]. The inflammatory mediators in the TME include cytokines, growth factors, chemokines and chemokine receptors. Those inflammatory signaling molecules can be generated by tumor cells themselves or by other cells (*e.g.* MSCs), which attract MSCs to homing into tumor sites and contribute to maintenance of the dynamic TME [47,48]. Several *in vitro* and *in vivo* studies have reported that MSCs can increase CSCs population and enhance CSCs tumorigenicity in many tumor types, including human ovarian tumors [49], breast cancer [9], prostate cancer [50], glioma [51], and colorectal tumors [52]. The molecular and cellular mechanisms of MSC–CSC communication are being investigated but still remain to be fully demystified.

MSCs are able to migrate into tumor's niches in response to multiple signals. Once MSCs are recruited into tumor sites, they may be "educated" by the TME to change their naïve gene signature and acquire pro-metastatic function to evolve into tumor-associated MSCs (TA-MSCs) [2]. TME/TA-MSCs can potentially "educate" newly arrived MSCs to expand their numbers and to promote tumor growth. TA-MSCs may also be differentiated directly from cancer cells or CSCs within certain contexts. For example, glioma-associated-human MSCs (GA-hMSCs), one type of TA-MSCs, in most cases, are genetically distinct from the glioma stem cells (GSCs) by the analysis of whole-genome sequencing and, on rare occasion, rarely 10%, the GA-hMSCs may differentiate directly from the GSCs [51]. TA-MSCs with MSC-like properties have become apparent as contributory stromal cells in tumor initiation, progression and metastasis. TA-MSCs have the ability to promote CSC proliferation and to maintain tumor "stemness" in ovarian cancer [49,53], glioma [51], and gastric cancer [54]. Taken together, these data provide evidence that both MSCs and TA-MSCs play a more potent pro-tumorigenic role in maintaining cancer stemness through increasing CSCs population.

MSCs: possible cell-of-origin of sarcoma

There is mounting evidence that suggests a possible relationship between the transformed MSCs and undifferentiated sarcoma. Kaposi's sarcoma-associated herpesvirus infection reprograms human oral MSCs with altered gene expression profile and transforms MSCs to Kaposi's sarcoma-like cells through mesenchymal-to-endothelial transition [55]. The *SSX* genes belong to a family of human CT antigens and the *SYT-SSX2* fusion gene is the synovial sarcoma-associated oncogene [56]. Reprogramming of MSCs by the *SYT-SSX2* leads to the aberrant differentiation of MSCs, as the first step toward transformation [56]. Importantly, TICs or CSCs have been identified in several types of sarcoma such as osteosarcoma [57], synovial sarcoma [58], and Ewing's sarcoma [59], contributing to drug-resistant properties. Notably, CSCs identified and characterized in the primary sarcoma express the three canonical surface markers (CD105/CD90/CD73) and retain MSC differentiation potential into osteocytes, chondrocytes, and adipocytes *in vitro* [57,59]. Although there is uncertainty regarding the cell-of-origin for sarcoma, increasing evidence suggests MSCs as the origin of sarcomas including in liposarcoma [60], osteosarcoma [61], and clear cell sarcoma [62]. A variety of mechanisms of MSC transformation are summarized in Fig. 2. To date, there are still disagreements regarding the susceptibility of MSCs to spontaneous transformation when serving as the origin of sarcomas.

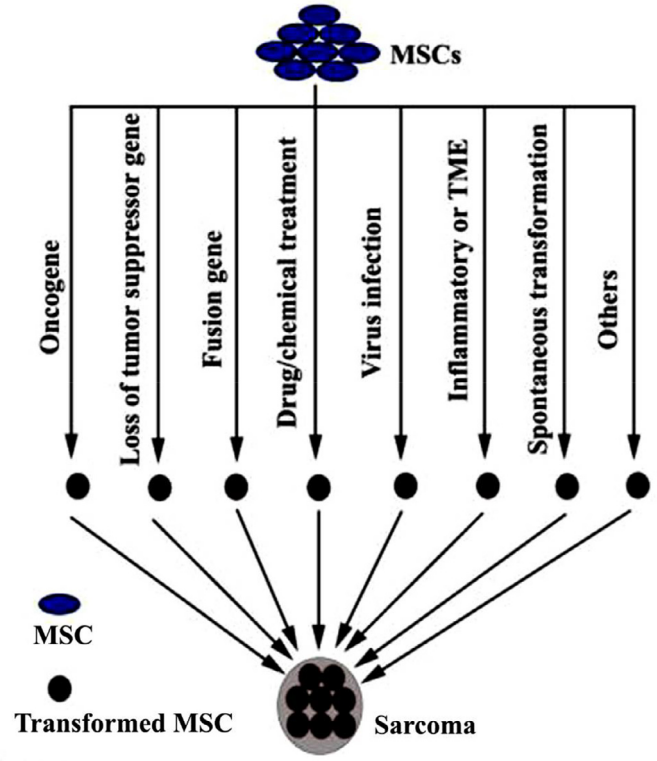

Fig. 2. Factors implicated in MSC transformation. There are different approaches to induce the transformation of MSCs, including over-expression of certain oncogenes (*e.g. Fos, RAS* and *hTERT*), loss of tumor suppressor genes (*e.g. P21* and *TP53*), expression of fusion genes (*e.g. FWS-FLI1, FUS-CHOP, SYT-SSX*), drug or chemical treatment, virus infection, inflammatory or tumor microenvironment (TME), spontaneous transformation and others. Combination approaches have been frequently applied to transform MSCs *in vitro* and *in vivo* studies.

Collectively, the different contributions of MSCs to tumorigenesis exist: (i) induction of EMT to generate CSC-like state in cancer cells; (ii) maintenance of cancer stemness; and (iii) potential to undergo malignant transformation and likely progenitors of certain tumorigenic cells (*e.g.* sarcoma). These prominent models of MSC-associated tumorigenesis proposed from current knowledge are summarized in Fig. 3.

Exploring MSC-based cancer therapeutics

The nature and relation of MSCs and CSCs are fundamental for tumorigenesis and cancer stemness. Given that MSCs can play a dual role in tumor growth and inhibition, MSC therapy in tumor is actively controversial. Safety issues regarding cancer recurrence caused by MSCs have also received the most attention in MSC-based anti-cancer therapy. Previous reports have suggested that MSCs might promote potential cancer recurrence [63,64], assuming that CSCs drive the tumor growth. Therefore, an in-depth understanding of the fairly unique properties of MSCs is important in enacting safe and effective therapies.

MSC-mediated tumor drug resistance

Cancers acquire a variety of damaging functional capabilities to attack on the affected individual and cancer cells develop resistance strategies under attack [65]. Cancers are comprised of a very heterogeneous population of cells and cancer cells can acquire resistance to the anti-cancer drugs. In this regard, targeted therapies are generally not very curative. Drug resistance can arise within cancer cells due to genetic changes (intrinsic resistance) or from the TME protecting cancer cells

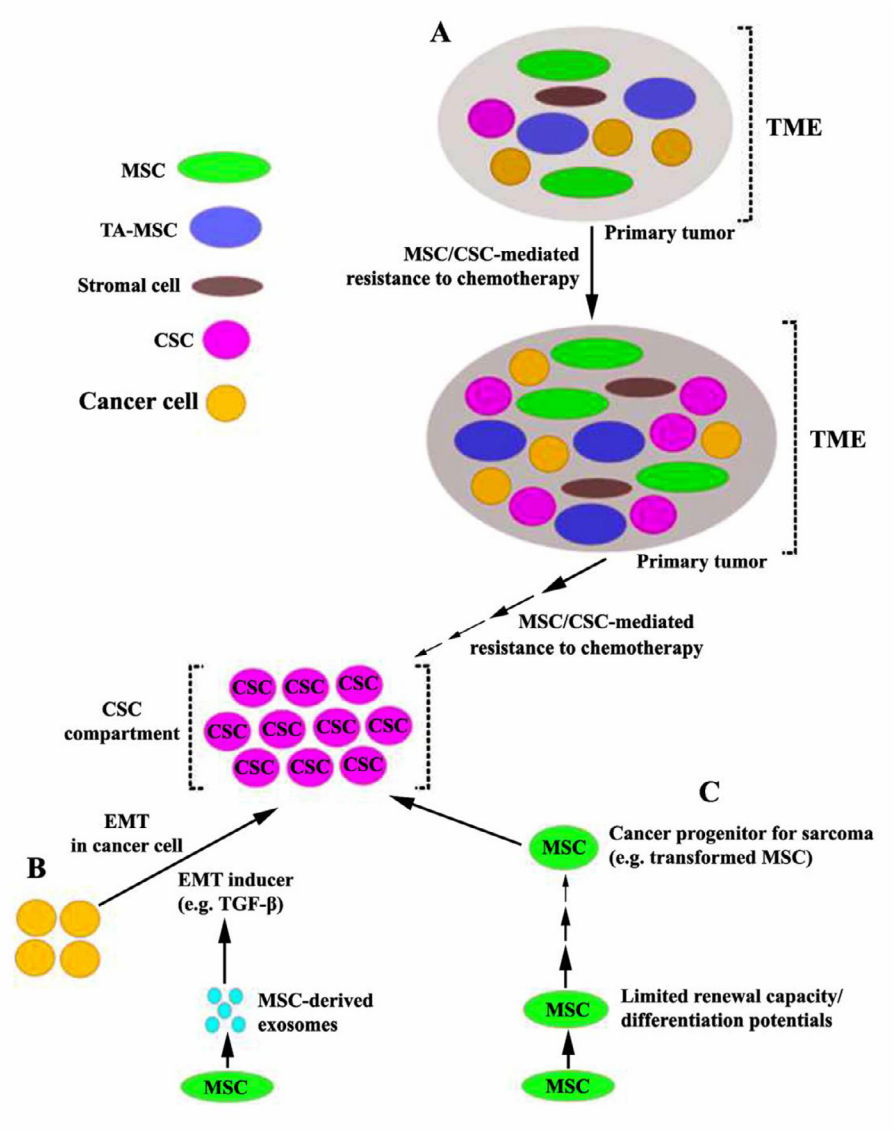

Fig. 3. Models of MSC-associated tumorigenesis. (A) Primary tumor is composed of various tumor cell subpopulations and tumor-associated stroma, including cancer stem cells (CSCs), mesenchymal stem cells (MSCs), tumor-associated MSCs (TA-MSCs), and other stromal cells. MSCs are "educated" by tumor microenvironment (TME) to reprogram into TA-MSCs [2]. Anti-cancer therapy may not eradicate all cancer cells due to MSCs/CSCs-mediated compound resistance. MSCs/TA-MSCs increase CSCs population and maintain cancer stemness. Thus, CSCs and MSCs sustain cancer cell growth and drive cancer relapse once and again. (B) MSCs-derived extracellular vesicle (*e.g.* exosome), carrying a variety of substance (*e.g.* TGF-β), can directly induce the epithelial-mesenchymal transition (EMT) in the cancer cells to generate the new CSCs. (C) MSCs have limited proliferative capacity and multi-differentiation potential. MSCs might be transformed due to abnormal genetic events. Transformed MSCs probably develop into cancer progenitor cells and increase CSCs populations.

against treatment (extrinsic resistance) [66]. Emerging evidence indicates that MSCs also have an important role in drug resistance [53,67], being likely responsible for persistent tumor recurrence. MSC-mediated resistance to chemotherapy in tumors could affect clinical response [68]. MSCs that are recruited into the tumor-associated stroma reside in close proximity to TICs in response to chemotherapy to cause drug resistance [48]. Multiple signaling pathways work together to promote resistance to anti-cancer drugs through MSC-TIC crosstalk [47]. CSCs have been identified for years in a variety of solid tumors, contributing to tumorigenesis, cancer development and chemoresistance [69,70]. Human MSCs have drawn increasing attention for exploring as new clinical therapeutic agents that are able to evade tumor drug resistance.

Optimum strategies for MSC therapy approaches

To date, there are a small number of registered clinical trials for exploring the capability of MSC-based anti-cancer therapies. A phase I clinical therapeutic trial has been conducted with allogeneic BM-MSCs to assess the safety and cancer-homing ability of MSCs into the foci of primary prostate cancer tissue [71]. This clinical study conducted by Schweizer et al. indicates that MSCs do not home to tumor sites in sufficient levels to guide further development as a therapeutic MSC-based delivery strategy, albeit systemically infused allogeneic MSCs are safe in patients with prostate cancer. Too little is currently known about MSC homing to specific sites *in vivo*.

Natural MSCs have intrinsic inhibitory effects on tumor growth, but harnessing this capacity remains under debate. Suppression of tumor growth by MSCs has received much attention in several cancer types, such as melanoma [72], pancreatic cancer [73] and breast cancer [74]. Although MSCs have the innate tumoritropic properties, the disadvantages of using MSCs for anti-cancer therapies involve the lacking of the selectivity and uncertainty in the aftermath, which often results in low efficiency and presents potential side effects. Genetically modified MSCs can effectively inhibit the growth of tumor cells. For example, the intraperitoneal injection of both MSCs and MSCs with interferon (IFN)-β exhibits the suppressive effects on pancreatic carcinoma xenografts in an orthotopic tumor model [73]. Importantly, this preclinical study also exhibits that MSCs-IFN-β treatment is more effective than innate MSCs treatment option.

Utilizing MSCs as potential carriers for a single agent against cancer may also have limitations, such as compound resistance and insufficient efficacy. For example, the engineered MSCs with tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) have shown the particular anti-cancer potential under preclinical settings. Cancer cells and

CSCs have resistance to TRAIL-induced apoptosis that frequently leads to the low therapeutic efficiency. Recombinant TRAIL (rTRAIL) formulations have been developed in order to increase the stability of TRAIL. One preclinical study has described that the rTRAIL secreted from human MSCs in combination with lipoxygenase inhibitor MK886 (MSCs-rTRAIL-MK886) results in significantly enhanced apoptosis of glioma cells compared to each agent alone [75]. These synergetic therapies with TRAIL-modified MSCs can overcome TRAIL-resistance in anti-cancer therapies. Another preclinical study reveals a combinatory approach between MSC-delivered sTRAIL (a secretable variant of TRAIL) and Paclitaxel (PTX) (MSCs-sTRAIL-PTX) as an efficient therapeutic tool for pancreatic adenocarcinoma [76]. As aforementioned, application of MSC-based synergistic combination approaches can enhance MSC-mediated anti-cancer efficacy and specificity.

MSC-based combination approaches for cancer therapy

Currently, MSCs have been genetically modified for the targeted delivery of various anti-cancer biological agents/compounds such as cytotoxic agents (*e.g.* TRAIL) and suicide gene therapeutic products. Oncolytic virus thymidine kinase (TK), cytosine deaminase (CD) and Carboxylesterase 2 (CE2) are been exploring for MSC-mediated suicide gene therapies, which opens new ways of MSC-based anti-cancer approaches.

There are different strategies for MSC-based anti-cancer synergistic combination approaches. One specific strategy is to create engineered MSCs with the oncolytic virus that simultaneously secrete different therapeutic agents, such as herpes simplex virus TK (HSK-TK) that converts a prodrug into a cytotoxin, selectively targeting tumor stroma microenvironment. For example, the application of MSCs transfected with the *HSV-TK* gene and the C–C chemokine ligand (CCL5) promoter (MSCs-CCL5-HSV-TK) combined with Ganciclovir (GCV) can significantly suppress pancreatic cancer growth [77]. The similar strategy is used by combining HSV-TK therapy with GCV (MSCs-HSV-TK-GCV) in rat leptomeningeal glioma model [78] and anti-PD-L1 (MSCs-HSV-TK-anti-PD-L1) in mouse melanoma brain metastasis model [79]. Tie2, an angiopoietin receptor, plays a critical role in the angiogenic process [80]. Such engineered MSCs with Tie2/HSV TK in combination with GCV serve as a Trojan horse targeting tumor stroma microenvironment under the selective control of the Tie2 promoter [81,82].

As noted above, another efficient MSC-based therapeutic strategy is to explore the optimized variants of TRAIL or TRAIL receptor agonists in combination of other different agents/drugs to overcome the resistance of cancer cells to TRAIL-based pro-apoptotic therapies. For example, the application of MSCs-dTRAIL-TK approach followed by GCV administrations often results in the inhibition of tumor growth and the prolonged survival of treated animals [83,84]. The application of MSC-based oncolytic virotherapy approach with TRAIL increases antitumor efficacy in brain tumor therapy [85]. More recently, a novel suicide gene, namely, iCasp9, has been proposed and MSCs co-expressing iCasp9 and TRAIL successfully target an aggressive sarcoma type [86]. Additionally, to enhance the homing capacity to treat glioblastoma, human MSCs with TGF-β pre-treatment and expression of a SMAD4-controlled promoter (MSCs-SBE4-TRAIL+TGF-β) exhibit high tumoritropism capacities and great curative efficacy [87]. Given the tumor-specific single-chain Fv antibody fragment (scFv), MSCs engineered as vehicles of scFvCD20-sTRAIL selectively migrate to the site of non-Hodgkin's lymphoma in a xenograft model and cause a significant increase of cellular apoptosis through both extrinsic and intrinsic apoptosis pathways [88].

Finally, bioengineered MSCs for targeted enzyme-prodrug therapy against cancer pave a new way to translational medicine. Engineered MSCs can express a suicide gene encoding an enzyme such as CD that converts 5-fluorocytosine (5-FC) to the toxic 5-fluorouracil [89]. Using CD-5-FC system in modified MSCs is therefore another promising specific strategy that can concurrently inhibit tumor growth. For instance, genetically modified MSCs to stably express yeast CD (CDy) in combination with daily administration of 5-FC (MSCs-CDy::UPRT+5-FC) increase therapeutic efficacy in killing mouse xenograft tumors without causing harm to normal tissues [90].

MSC-based treatment for targeting CSCs

Due to CSC-mediated resistance to chemotherapy and likely recurrence of malignant tumors, exploring MSCs delivery capability for targeting CSCs is an important therapeutic goal. To date, limited studies are focused on MSCs' ability to migrate toward CSCs. One preclinical study is conducted with targeting TICs using the nano-ghosts derived from MSC membranes and a C-X-C receptor 3 (CXCR3) antagonist in combination with Gemcitabine, resulting in effectively eliminating TICs and overcoming compound resistance [48]. TGF-β-mediated homing of BM-MSCs carrying the oncolytic adenovirus, Delta-24-RGD, to GSCs shows the effective treatment for glioblastoma by targeting GSCs [91]. In an intracranial xenograft model of TRAIL resistant primary GSCs, engineered MSCs to express sTRAIL in combination with low dose Cisplatin are more effective in the inhibition of tumor growth and prolonged mouse survival [92]. Additionally, MSCs infected with the engineered oncolytic adenoviruses, wherein the *E1B19K* gene is deleted to increase viral release or a *TRAIL* transgene is inserted, demonstrate an effective delivery in tumorigenic cells with CSC characteristics in xenotransplantation studies [93]. Importantly, the virus capsid protein that reflects the amount and location of viruses can be detected in tumor xenografts, but not in normal tissue [93].

CE2 converts a prodrug irinotecan into its cytotoxic form (SN-38), the potent topoisomerase inhibitor [89]. Human MSCs are genetically engineered to express recombinant secretory human CE2 (shCE2) and nanoluciferase genes for the targeted enzyme-prodrug therapy of ovarian cancer intraperitoneal metastasis [94]. Through a metabolomics approach, the new therapy delivers the drugs to both tumor supporting cells in tumor stroma and cancer stem-like cells in necrotic/hypoxic regions [94]. BMP4, a part of the TGF-β superfamily, has been shown to inhibit tumor-initiating capacity [95]. One preclinical study has reported that non-virally engineered human MSCs with biodegradable polymeric nanoparticles (NPs) to deliver BMP4 (MSCs-PBAEs NPs-BMP4) have a novel therapeutic effect of specifically targeting human brain TICs [96]. Importantly, this approach overcomes the blood-brain barrier after both intranasal and systemic intravenous administration and the homing efficiency is raised in the brain xenografts [96].

While clinical studies exploring MSC therapy have been conducted for decades with an increasing number of trials, the therapeutic benefit of MSCs has fallen short of expectations in most clinical trials. The key challenges of MSC-based therapeutic products are to achieve their safety and effectiveness in preclinical observations and in clinical trials. Safety and efficacy data collected from preclinical and clinical studies supporting MSC-based anti-cancer therapies and combination approaches using MSCs therapeutic products are exemplified in Table 1.

Clinical challenges and opportunities

Currently, the MSC-based anti-cancer approaches have not yet been widely adopted into clinical practice, due to uncertainty of clinical response rates and anti-tumor activity. For example, one clinical study has reported that twelve children with neuroblastoma were treated with a high dose of MSCs with oncolytic adenovirus, and of those, less than 50% had a positive clinical response to treatment [97]. Exploring MSC-based anti-cancer therapeutics is currently ongoing based on the innate ability of MSCs to home into TME. MSCs can be considered as the potential carriers for delivery of therapeutic agents directly to tumor sites. The MSC-based delivery system proposes to target TICs and overcome compound resistance. However, the precise mechanisms of action of MSCs are unclear and a series of potential challenges have to be addressed for more safe and effective MSC-based therapies.

Firstly, one key issue towards clinical translation is the fate of MSCs after systemic infusion. Little is currently known about long-term fate

Table 1
A summary of MSC-based anti-cancer combinatory approaches.

Source	Combination	Tumor/CSCs type	Effect	Reference
MSC-based combination therapies				
BM-mMSCs	MSCs-CCL5-HSV-TK+GCV	Pancreatic cancer	Suppression	[77]
BM-rMSCs	MSCs-HSV-TK+GCV	Glioma	Suppression	[78]
hMSCs	MSCs-HSV-TK+anti-PD-L1	BMM	Suppression	[79]
BM-mMSCs	MSCs-Tie2-HSV-TK+GCV	Pancreatic cancer	Suppression	[81]
BM-mMSCs	MSCs-Tie2-CCL5-HSV-TK+GCV	Hepatocellular carcinoma	Suppression	[82]
BM-rMSCs	MSCs-dTRAIL-TK+GCV	Renal Cell Carcinoma	Suppression	[83]
BM-hMSCs	MSCs-sTRAIL-HSV-TK+GCV	Glioblastoma multiforme	Suppression	[84]
BM-hMSCs	MSCs-HSV-TRAIL	Glioblastoma multiforme	Suppression	[85]
AD-hMSCs	MSCs-TRAIL-iCasp9	Ewing Sarcoma cells	Suppression	[86]
AD-hMSCs	MSCs-SBE4-TRAIL+TGF-β	Glioblastoma multiforme	Suppression	[87]
UC-hMSCs	MSCs-scFvCD20-sTRAIL	B-cell lymphoma	Suppression	[88]
BM-hMSCs	MSCs-CDy::UPRT+5-FC	Xenograft ovarian tumor	Suppression	[90]
MSCs targeting CSCs applications				
BM-hMSCs	MSCs-NG-AMG487+GEM	TICs in Pancreatic cancer	Apoptosis of TICs	[48]
BM-hMSCs	MSCs-ADV+ TGF-β	GSCs	Suppression	[91]
AD-MSCs	MSC-sTRAIL+cisplatin	GSCs	Apoptosis of GSCs	[92]
BM-hMSCs	MSC-ADV5/3-TRAIL	CSCs in PDA	Suppression	[93]
AD-MSCs	MSC-shCE2+Irinotecan	CSCs in ovarian cancer	Targeting CSCs	[94]
AD-hMSCs	MSC-PBAEs NP-BMP4	BTICs	Suppression	[96]

AD-hMSCs: human adipose-derived MSCs; ADV: adenovirus; AMG487: CXCR3 antagonist; BM-hMSCs: human BM-MSCs; BM-mMSCs: mouse BM-MSCs; BM-rMSCs: rat BM-MSCs; BMM: brain metastatic melanomas; BMP4: bone morphogenetic protein 4; BTICs: brain tumor initiating cells; CCL: C–C chemokine ligand; CDy::UPRT: cytosine deaminase::uracil phosphoribosyltransferase; CE2: carboxylesterase-2; CSCs: cancer stem cells; dTRAIL: dodecameric TRAIL; Fu: 5-fluorouracil; GCV: ganciclovir; GEM: Gemcitabine; GSCs: glioma stem cells; HSV: herpes simplex virus; iCasp9: a novel suicide gene; NG: Nano-ghost; NP: nanoparticle; PBAEs: poly(beta-amino ester)s; PDA: pancreatic ductual adenocarcinoma; SBE4: SMAD4-controlled minimal promoter; scFv: single-chain antibody; shCE2: recombinant secretory human CE2; s-TRAIL: secretable variant of TRAIL; TGF-β: transforming growth factor-β; Tie2: an angiopoietin receptor; TICs: tumor-initiating cells; TK: thymidine kinase gene; UC-hMSCs: human umbilical cord-derived MSCs.

of MSCs. One previous study following MSCs therapy in human using clinical autopsy tissue samples indicated that detection of MSC donor DNA was negatively correlated with time from infusion to sample collection, suggesting that MSCs appear to exert their function via the "hit and run" mechanism [98]. This raises the possibility that most of circulating MSCs may be cleared from the body by the immune system after systemic infusion and very limited number of remnant MSCs migrates to other tissues in the body. In one previous pre-clinical study, Shi et al. traced the mobilization and localization of intravenously transplanted human umbilical cord mesenchymal stem cells (UC-MSCs) for treatment of rat diabetic foot ulcer, one of the most common diabetic complications [99]. This study by Shi et al. showed that the distribution of UC-MSCs were detected in the wound tissues, but not very large, creating a biological microenvironment to accelerate healing through paracrine mechanism, angiogenesis and transdifferentiation. Therefore, new pre-clinical studies should be focused on the molecular mechanisms of signaling pathways in response to the fate of MSCs after administration.

Secondly, another key issue in terms of safety is the malignant transformation of transplanted MSCs. MSCs culture expansion *in vitro* is often required for clinical or experimental studies. Critical factors that can profoundly influence the behavior of MSCs include donor age, isolation method, culture conditions and repeated passaging. In addition, neoplastic transformation risk should be determined in the genetically engineered MSCs during the conduction of therapeutic approaches. To date, there are no preclinical studies reports describing any significant adverse events of the modified MSCs under different experimental settings. However, it is important to be aware whether gene-modified MSCs may be detrimental to MSC-based therapies.

Thirdly, it still maintains unclear whether MSCs have the sufficient capability of homing and engraftment for tissue targeting *in vivo*. Novel methods to modify MSCs for enhanced targeting are more likely required. Applications of engineered MSCs with overexpression of the key molecules such as chemokines/chemokine receptors and adhesion molecules can promote the tumor tropism. However, *in vitro* experiments on homing capability of MSC are inconclusive because they may not completely reflect the "real milieu" of MSC homing *in vivo*. In our recent Commentary to the Drug Design, Development and Therapy, we critically analyzed and addressed the variability of MSC properties that may influence MSC homing capability, such as the high culture confluence, loss of expression of some surface receptors, and cellular stress conditions [100].

Fourthly, the inherent MSC properties may be critical for successful clinical translation, such as MSC tissue source, donor age, isolation, propagation, cryopreservation and thawing. For example, MSC isolation is universally achieved either by enzymatic digestion or by the explants (mechanical dissociation) culture method. Enzymatic digestion, typically using collagenases and hyaluronidases, is frequently applied to isolate MSCs from various tissues. While the lack of standardized methods employed for MSC isolation, experimental protocols for isolation of MSC from different tissue sources are different regarding yield, efficiency and cell quality, such as enzyme or enzyme cocktail, enzyme concentration, digestion time and buffers, centrifugation speed and mesh filter size [101]. Additionally, other multiple parameters, including medium composition, serum supplementation, O_2 and CO_2 concentration, pH and temperature, can vary the exact properties of MSCs during MSC expansion and preparation [101,102].

Fifthly, the heterogeneity in the TME can actively influence therapeutic response. The interactions between MSCs and various immune cell types in the context of cancer, including T cells, B cells, natural killer (NK) cells, macrophages and dendritic cells, can both inhibit and favor tumor growth through cell-cell contact and/or a paracrine mechanism [103]. While MSCs have been extensively reported their immunosuppressive functions (*e.g.*, immunosuppressive effects on NK cells), their diverse immunomodulatory properties are been exploiting. For example, the differential immunomodulatory effects of MSCs on NK cell lines, KHYG-1 and NK-92, in one previous study by Hu et al. indicated that, unlike KHYG-1, the killing activity of NK-92 cells against K562 targets was not affected by MSCs when NK lines were co-cultured with MSCs

[104]. The crosstalk between MSCs and immune cells may guide an antitumor strategy for the applications of the combined cell therapeutic approaches. In a follow-up study, Moreno et al. showed that the combination of OAdv (an oncolytic adenovirus)-loaded menstrual blood derived MSCs and allogeneic peripheral blood mononuclear cells enhanced antitumor efficacy both *in vitro* and *in vivo* [105]. The combined cell therapy may improve antitumor treatment outcome, which holds promising therapeutic potential.

Finally, there are still important issues to address for optimizing MSCs therapeutic regimens that need to be determined in clinical trials, including the appropriate dose of MSCs, dosing strategy of MSCs, the route of infusion, and the timing of MSC administration.

Conclusions and perspectives

Given the bi-directional effects on tumor growth and inhibition, MSCs may also potentiate tumorigenesis, tumor growth and metastasis after systemic infusion. Therefore, MSC products should be used with great caution for these patients suffering from certain forms of cancer. This review addresses that the risk factors for long-term adverse events should be carefully analyzed in clinical trials. There would be considerable skepticism regarding many claims made for MSC therapies. The bottom line, however, is that administered MSCs appear to be safe.

Due to cancer resistance to therapeutic agents, compared to anticancer monotherapy, using the synergistic combination therapies that address diverse aspects of cancer pathology can enhance MSC-mediated anti-cancer efficiency and specificity with improved safety profiles. Currently, phase I and II clinical trials using MSC-based anti-cancer treatment are already underway. For example, in one phase I/II clinical trial, TREAT-ME-1, genetically modified autologous MSCs (HSV-TK), in combination with GCV was confirmed safe and tolerable in patients with the advanced gastrointestinal cancer [106,107]. Another phase I/II clinical trial that is registered with ClinicalTrials.gov (NCT03298763) is underway to assess the safety and synergistic anti-tumor efficacy of TRAIL-expressing MSCs combined with prodrugs Cisplatin and Pemetrexed in lung cancer patients.

To develop new MSC-based anti-cancer therapy, much more needs to understand the underlying cellular and molecular mechanisms of action of MSCs. Prospective research should further deliberate how well the therapeutic delivery can be paired to the tumor properties, such as tumor type, tumor location, tumor stage, compound resistance, tumor growth and metastasis, for more effective and safe MSC-based anticancer therapies.

Declaration of Competing Interest

The authors declare no competing interests for this work.

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Author contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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