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The Oncogenic Potential of Mesenchymal Stem Cells in the Treatment of Cancer: Directions for Future Research

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Abstract

Mesenchymal stem cells (MSCs) represent a promising new approach to the treatment of several diseases that are associated with dismal outcomes. These include myocardial damage, graft versus host disease, and possibly cancer. Although the potential therapeutic aspects of MSCs continue to be well-researched, the possible hazards of MSCs, and in particular their oncogenic capacity are poorly understood. This review addresses the oncogenic and tumor-supporting potential of MSCs within the context of cancer treatment. The risk for malignant transformation is discussed for each stage of the clinical lifecycle of MSCs. This includes malignant transformation *in vitro* during production phases, during insertion of potentially therapeutic transgenes, and finally *in vivo* via interactions with tumor stroma. The immunosuppressive qualities of MSCs, which may facilitate evasion of the immune system by a tumor, are also addressed. Limitations of the methods employed in clinical trials to date are reviewed, including the absence of long term follow-up and lack of adequate screening methods to detect formation of new tumors. Through discussions of the possible oncogenic and tumor-supporting mechanisms of MSCs, directions for future research are identified which may eventually facilitate the future clinical translation of MSCs for the treatment of cancer and other diseases.

Keywords

mesenchymal stem cells; stem cell transplantation; oncogenesis

INTRODUCTION

Mesenchymal stem cells (MSCs) have received considerable attention in recent years for several potential therapeutic applications, including myocardial tissue repair, prevention of graft versus host disease, and the treatment of cancer. While the therapeutic promise of MSCs has been reiterated time and again in multiple reviews, the potential hazards of their use are infrequently addressed. This review considers the oncogenic potential of MSCs, with a focus on the use of MSCs to treat cancer. Although almost all animal studies that have employed genetically-modified MSCs for the treatment of cancer have shown therapeutic effects [1], an understanding of oncogenic mechanisms that may give rise to cancers in humans over an extended time frame will be necessary to facilitate the clinical translation of MSCs. The approach in this review is to describe how MSCs may undergo malignant transformation at each phase of their clinical lifecycle, from initial isolation, to expansion in culture, transfection

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with therapeutic transgenes, and finally, administration to patients. Additionally, the immunosuppressive properties of MSCs, which may promote evasion of the immune system by tumors, are also addressed. By identifying these potential oncogenic and tumor-enhancing mechanisms, we define areas for further research which we hope will facilitate the translation of MSCs to clinical use for the treatment of cancer.

DEFINITION OF A MESENCHYMAL STEM CELL

MSCs are classically defined by the initial experimental protocols that were used to isolate them from bone marrow [2,3]. Bone marrow aspirates can be dissociated into a suspension containing hematopoietic stem cells and marrow stromal cells. The hematopoietic stem cells give rise to erythroid, lymphoid, and myeloid progenitors; whereas the marrow stromal cells support hematopoiesis, comprise the structural matrix of bone marrow, and are capable of differentiating into the osteogenic, chondrogenic, and adipogenic lineages. When cultured, only the marrow stromal cells adhere to the flask, and it is possible to separate these cells by repeatedly changing the media. These adherent cells were termed *colony-forming unit fibroblasts* due to their fibroblast-like morphology and the propensity to form colonies in culture [3]. They are now referred to as *mesenchymal stem cells* or *marrow stromal cells*. Their characteristic morphology is shown in Fig. (**1**).

The immunophenotypic definition of what constitutes an MSC has only recently been standardized. The minimal criteria that define an MSC, as set forth in 2006 by the International Society of Cellular Therapy (ISCT) are:

1. Plastic-adherence in standard culture conditions;
2. Expression of the mesenchyme markers CD105, CD73 and CD90, and no expression of markers of contaminating endothelial, hematopoietic, or immunological cells (CD45, CD34, CD14 or CD11b, CD79α, or CD19 and HLA-DR surface markers); and
3. The ability to differentiate into osteoblasts, adipocytes and chondroblasts [4,5].

There is no one marker that is specific for MSCs, and combinations of markers must be specified to distinguish cell types with behavior characteristic of MSCs. Although the ISCT definition will help to standardize future research, most of the existing studies published since the initial isolation of MSCs in 1970 have used inconsistent defining characteristics of MSCs, which may in part explain the high prevalence of conflicting experimental results. This inconsistency could call into question the validity of many of the experiments that have used these cells, and at worst, may influence the results of clinical trials [6].

IN VITRO MALIGNANT TRANSFORMATION

Considerable *in vitro* expansion is often necessary to achieve adequate numbers of MSCs for therapeutic purposes [7–9]. This *in vitro* expansion phase is the first point at which MSCs become susceptible to malignant transformation, as shown in Fig. (**2a**). Rubio *et al*. showed that human adipose-derived MSCs undergo spontaneous transformation after 4–5 months of culture, through the sequential upregulation of *c-myc* and downregulation of p16, although this phenomenon was not observed after only 6 to 8 weeks in culture [10]. Wang *et al*. noted that the *in vitro* culture of human bone marrow-derived MSCs produces a sub-population of cells with high levels of telomerase activity, chromosomal aneuploidy, and translocations, that are capable of forming tumors in multiple organs in NOD/SCID mice [11]. These findings were not reproduced in a subsequent study, in which chromosomal abnormalities were absent, and normal telomere shortening was observed, in human bone marrow-derived MSCs that were propagated to senescence or 25 passages [12]. Because the results of such experiments conflict

with one another, and because available research on *in vitro* characteristics of MSCs is limited, the possibility of malignant transformation *in vitro* remains highly controversial. Future studies which employ standardized isolation protocols for MSCs will therefore be needed to elucidate the poorly understood potential for malignant transformation during the *in vitro* expansion phase [13].

MESENCHYMAL STEM CELLS MIGRATE TO TUMORS

In vivo experimental studies on the migration of MSCs are summarized in Table 1 and Table 2. These tables include several supporting clinical studies that have rigorously tracked the distribution of MSCs administered to patients. Taken together, these studies demonstrate two important *in vivo* characteristics of MSCs: MSCs migrate toward tumors, but this migration is non-specific [1,13]. The migratory tropism toward tumors has been observed when MSCs are administered by intravenous [14], intraarterial [15], or peritumoral routes [16]. The mechanism of migration is poorly understood, but has been shown to be dependent upon the cytokine/receptor pairs SDF-1/CXCR4 [15,17,18], SCF-c-Kit [19,20], HGF/c-Met [21], VEGF/VEGFR [22], PDGF/PDGFr [15], MCP-1/CCR2 [23], and HMGB1/RAGE [24,25], as well as cellular adhesion molecules [18,26,27]. Migration to tumors, however, is non-specific as exogenously administered MSCs have also been shown to localize to the lung [14,28–32], bone marrow [29,30,33,34], and lymphoid organs [35,36]; and prior whole body irradiation tends to expand the distribution of MSCs in the body to multiple organs [28,30]. Additionally, MSCs appear to migrate to sites of localized chronic inflammation [35,37], which may in part explain the observation that MSCs are recruited in the process of wound repair [38–42]. Although MSCs have been shown to enhance metastatic potential in an animal model of breast carcinoma [43], there have been no reports of tumor seeding by MSCs in normal, non-cancerous tissue. These interactions between MSCs and normal, noncancerous tissue, however, are probably very different from the interactions of MSCs with abnormal, neoplastic tissue. These interactions between MSCs and cancerous tissue have been researched more thoroughly, and are addressed in subsequent sections of this review.

IMMUNOSUPPRESSION BY MESENCHYMAL STEM CELLS MAY FAVOR TUMOR GROWTH

In several animal tumor models, including melanoma [44,45], colon adenocarcinoma [46], multiple myeloma [47], lung cancer [48], and glioblastoma [48], the presence of exogenous MSCs was shown to enhance tumor formation. Such studies provide indirect evidence that there may be a cancer-promoting interaction between MSCs and tumors. One potential mechanism underlying these observations is immunosuppression. MSCs exert an immunosuppressive effect by interacting with almost all cells of the innate and adaptive immune systems, and these interactions may enhance the ability of some tumors to evade immune surveillance [13].

In the adaptive immune system, MSCs interact with both T-cells and B-cells. The effects of MSCs upon T cells are two-fold. First, MSCs tend to support the survival of T cells that are in a quiescent state. T cells that are exposed to MSCs are arrested at the G1 phase of the cell cycle, in a state that resembles division arrest anergy [49]. This process may be dependent on inhibition of cyclin D2 and the upregulation of CDKN1B [49]. Furthermore, MSCs protect unstimulated T cells from activation induced cell death through downregulation of the Fas ligand and receptor [50].

Second, MSCs suppress proliferation of T-cells that are in an activated state. This T cell suppression occurs by one of three mechanisms. The first mechanism involves the concerted action of IFN γ with one of 3 pro-inflammatory cytokines: TNF α, IL-1α, or IL-1β [51]. IFN

γ causes T cells to produce the enzyme indoleamine 2–3 dioxygenase (IDO) [51–53]. IDO is important because it depletes the essential amino acid tryptophan, which is required for lymphocyte proliferation. The pro-inflammatory cytokines TNF α, IL-1α, and IL-1, cause MSCs to secrete iNOS and the chemokines CXCL-9 and CXCL-10 [51]. The elevated expression of iNOS results in high local levels of nitric oxide, which inhibits T lymphocytes in part by suppressing STAT-5 phosphorylation [54]. The chemokines attract T cells into proximity with MSCs [51]. These T lymphocytes which have migrated to a microenvironment that includes high levels of IDO and nitric oxide, then become suppressed. Therefore, MSCs suppress the function of T cells via IFN γ and pro-inflammatory cytokines. The second mechanism of T cell suppression by MSCs involves the non-classical human leukocyte antigen (HLA) class I molecule HLA-G5, which has been shown to suppress T-cell proliferation and increase production of T regulatory cells [55–57]. When MSCs make physical contact with stimulated lymphocytes, they are capable of secreting HLA-G5 in an IL-10-dependent manner [56]. Finally, T cells are indirectly suppressed by the actions of MSCs on dendritic cells (DCs), which are described below. When T cells are suppressed by MSCs, they shift to an anti-inflammatory state. This anti-inflammatory state is characterized by decreased IFN γ production by TH1 cells [57], decreased production of IL-4 production by TH2 cells [57], and diminished production of TNF α [35]. Therefore, MSCs suppress T cells through the actions of IFN γ, HLA-G5, or the suppression of DCs, causing a shift to an anti-inflammatory state.

Direct suppression of B cells by MSCs may occur to a limited extent [58]. As with T cell suppression, a mechanism involving arrest at the G0/G1 phase appears to be involved in B cell suppression, which impairs production of IgM, IgG, and IgA [59]. Cell surface interactions, including the engagement of PD-1 receptor, are also necessary for this effect [60]. Direct suppression of B cells by MSCs remains controversial, as other studies have demonstrated conflicting results [52]. Most likely, the dominant mechanism for the suppression of B cells is indirectly through suppression of T-cells.

MSCs suppress the innate immune system primarily through their effects on dendritic cells (DCs). Dendritic cells process antigenic material, mature, and then function as antigen presenting cells to naive T lymphocytes. MSCs inhibit three critical functions of dendritic cells: maturation, antigen presentation, and secretion of pro-inflammatory compounds. MSCs inhibit the maturation of DCs, or more specifically, the commitment of CD 34+ cell-derived [61] and monocyte-derived precursors [61–64] to differentiate into DCs. This inhibition occurs by blocking DC precursors from entering the G1 cell cycle phase, and through the downregulation of cyclin D2 [62]. Furthermore, activation of the Notch signaling pathway appears to be involved, as the administration of inhibitors of Notch signaling to cocultures of MSCs and DC precursors reverses the effects of MSCs on DC cell maturation [65]. MSCs also inhibit antigen presenting functions by DCs [62]. Co-culture of MSCs with DCs or DC precursors results in diminished levels of cell surface molecules associated with antigen presentation, including MHC class II, CD1a, CD40, and CD 86 [63,64]. Finally, MSCs suppress DC cell function by inhibiting their secretion of the pro-inflammatory compounds TNF α and IL-10 [57]. Therefore MSCs interact with dendritic cells to inhibit their maturation, antigen presentation, and secretion of pro-inflammatory compounds.

MSCs further suppress the innate immune system by acting on NK cells and neutrophils. The interactions between MSCs and NK cells represent an example of reciprocal inhibition. The activation of NK cells is highly dependent on cell surface receptors. These cell surface receptors allow NK cells to recognize target cells and are required for NK cell-mediated lysis to occur. The cell surface receptors NKp30, NKG2D, DNAM-1, and LFA1, which are present on the surface of NK cells, are activated by ligands that are present on the surface of MSCs: ULBP, PVR, Nectin-2, and ICAM-1, respectively [66,67]. Through their interactions with these cell surface receptors, activated NK cells are able to lyse autologous and allogeneic MSCs [66,

68]. However, MSCs exert an opposing effect by down-regulating the expression of NKp30 and NKG2D, ultimately inhibiting the cytotoxic activity of NK cells, cytokine production, and proliferation [68,69]. Furthermore, the HLA-G5 and IDO systems discussed previously also appear to inhibit NK cells [56,69]. MSCs act on both resting and activated neutrophils by dampening the respiratory burst [70]. However, MSCs also inhibit apoptosis of neutrophils through the IL-6 induced upregulation of STAT-3, and do not impair phagocytosis, or expression of adhesion molecules on neutrophils.[70] These effects on the immune system may contribute in part to tumor-enhancing properties of MSCs. However, a second factor—the interaction between MSCs and tumor stroma—likely plays a siginificant role. These interactions are discussed next.

MALIGNANT TRANSFORMATION FROM INTERACTIONS WITH TUMOR STROMA

A second mechanism that accounts for enhancement of tumor formation by MSCs is a possible transforming effect exerted by tumor stromal cells upon mesenchymal stem cells [71]. Tumors are comprised of a heterotypic array of malignant cells in communication with stromal cells. This tumor stroma is composed of endothelial cells, immune cells, and fibroblasts, which are thought to support the neoplastic properties of cancer cells [72–77]. Several studies suggest that MSCs could be a source of one type of stromal cell in breast cancer—the carcinoma-associated fibroblast (CAF), as shown in Fig. (**2b**) [71,74,78]. CAFs are a mixed population of myofibroblasts and activated fibroblasts which have been found to support growth of cells and angiogenesis in breast cancer [79]. Notably MSCs can assume a CAF-like phenotype after prolonged exposure to tumor-conditioned media [78]. These CAF-like MSCs possess a phenotype similar to CAFs, enhance tumor growth both *in vivo* and *in vitro*, and demonstrate sustained expression of the stromal-derived factor-1 (SDF-1) and the myofibroblast marker α-smooth muscle actin (α-SMA) [78,79]. Additionally, MSCs have also been shown to adopt a myofibroblast phenotype in response to transforming growth factor-β (TGF-β) [78,80]. Taken together, these observations have led some authors to believe that MSCs exposed to the local environment of a tumor could differentiate into CAFs and may therefore enhance tumor growth or spread [71].

Further understanding of such interactions between MSCs and tumor stromal cells will require more refined animal models than are currently available [81]. Standard xenograft models are of limited use in studying the interactions between MSCs and tumor stroma because they fail to fully capture the complexity of the tumor stroma as it occurs in patients [82,83]. Most animal tumor models are created by first culturing human cancer cells or cancer stem cells *in vitro* and engrafting the cells into an animal. During the *in vitro* culturing phase, cells lines adapt to and proliferate in culture conditions independent of contact with stromal and epithelial cells, and this process may eventually result in cells with a phenotype that differs markedly from that found in patients [82]. Once these cells are engrafted into an animal, the stroma that forms is derived from the animal's own cells, resulting in a chimeric tumor that does not precisely represent the human condition. Because human cellular signaling pathways differ from those of mice [84] and other animals, these chimeric tumors most likely do not reproduce the interactions that would occur between human MSCs and human tumor stroma. Animal models that more accurately recapitulate the tumor stromal characteristics observed in patients, and that are capable of engrafting human cell types that comprise the tumor stroma, are therefore required for definitive studies on the interactions of MSCs with tumor cells, and in particular, the stromal component. The new generations of immunocompromised mice, such as the NOG (NOD/Shi-*scid*/IL-2Rγ^{null}) mouse, which accept heterologous cell populations more readily than wildtype animals, represent one new advancement in this direction [85].

MIGRATION AND TUMOR FORMATION: PARALLELS WITH THE BRAIN TUMOR STEM CELL HYPOTHESIS

The notion that stem cells can migrate toward a tumor, develop into a malignant phenotype, and subsequently contribute to the tumor mass, parallels a broader line of reasoning which has been proposed to explain the origins of brain cancer. This line of reasoning is termed the *brain tumor stem cell (BTSC) hypothesis* [86–89]. The BTSC hypothesis proposes that oncogenic alterations in neural stem cells (NSCs), or NSC-like cells, may give rise to brain tumors [90]. Therefore, brain cancers represent clonally-derived tissues that arise from a single abnormal stem cell. The BTSC hypothesis arose from observations that normal, non-cancerous neurogenesis continues to take place in the adult brain [90–96]. These mechanisms of neurogenesis in the adult brain rely on NSCs which reside in 3 different niches—the subventricular zone of the lateral ventricles (SVZ), the subgranular layer of the hippocampal dentate gyrus, and the olfactory bulbs [97–99]. From these niches, NSCs most likely migrate into the brain parenchyma [100], and serve as precursors for new neurons, oligodendrocytes, and astrocytes. The BTSC hypothesis proposes that these normal mechanisms of neural development that stem from normal NSCs parallel the abnormal oncogenesis that results from abnormal NSCs.

The BTSC further proposes that the development of a brain tumor parallels normal adult neurogenesis by recruiting NSCs to the site of the tumor. In support of this claim is the observation that NSCs show extensive migratory tropism to brain tumors, to the extent that they can even be modified genetically to serve as gene delivery vehicles for the treatment of cancer [1,101]. Furthermore, the proximity of brain tumors to germinal areas such as the SVZ affects their clinical characteristics, suggesting that cells in these niches may affect the process of tumor formation [102,103]. In a recent series, Lim *et al.* found that 56% of GBM tumors in contact with the SVZ and cortex were multifocal, but tumors not in contact with the SVZ or cortex were not multifocal [102]. Furthermore, patients with GBM tumors that are not in close proximity to germinal zones survive 3 months longer from the time of diagnosis, than patients with tumors in close proximity to germinal zones [103]. These findings support the hypothesis that brain tumors recruit stem cells from germinal zones, such as the SVZ, which migrate to the tumor, and contribute to tumor formation.

We speculate that there are several parallels that remain to be explored between NSC and MSC migratory characteristics within the context of brain cancer. NSCs are recruited to a neural niche in gliomas, where they contribute to tumor formation. Similarly, it is possible that MSCs may be recruited to the stromal portion of tumors, which could serve as a mesenchymal niche, and thus contribute to tumor formation. As previously discussed, this stromal portion could favor malignant transdifferentiation of MSCs, which would in turn account for the oncogenic properties of MSCs documented in some studies [44–48]. Additional research is necessary to determine if any such parallels exist between the migratory characteristics of NSCs and MSCs.

MALIGNANT TRANSFORMATION FROM INCORPORATION OF TRANSGENES

The observation that MSCs migrate toward tumors has prompted a new approach to treating cancer in which genetically-modified MSCs serve as tumor-selective gene delivery vehicles (Table 2). The basic approach, which has only been employed in animal models thus far, calls for the harvest of MSCs, modification of the MSCs such that they secrete an anti-neoplastic compound, and finally administration of the MSCs into an animal possessing a tumor. Stem cells have already been genetically modified to secrete 11 different compounds with varying success in prolonging survival and reducing tumor mass (reviewed in Aboody *et al*. [1]).

Although this approach to treating cancer holds tremendous promise, the genetic modification of MSCs—or any cell type which is to be administered to patients—may also be hazardous.

The possible dangers of transgenic cell therapy stem from one of two possibilities: that either the transgene is tumorigenic, or that its insertion disrupts a genomic locus that is critical for tumor suppression, as shown in Fig. (**3**). The tumorigenicity of a transgene is of concern within the context of using genetic modification to immortalize stem cell lines. Incorporation of an immortalizing transgene may be necessary for therapeutic application of MSCs because MSCs have traditionally been derived from small tissue samples (usually bone marrow) that must be expanded to billions of cells in order to reach therapeutic levels. Significant expansion of an unmodified cell line, however, is difficult since MSCs become senescent after several passages [104]. Therefore the incorporation of immortalizing genes has been explored as an approach to maintain cell lines indefinitely [1]. Immortalizing transgenes include the proto-oncogene v-*myc* [101,105–110], human telomerase reverse transcriptase (hTERT) [111–118], human papillomavirus type 16 E6/E7 [119–122], Bmi-1 [123–129], and the N-terminal fragment of SV40 large T-antigen [1,130–133]. Notably, the potential tumorigenic capacity of these immortalized cells lines is not well-studied, and it is unknown whether loss of normal cell-cycle checkpoint controls, karyotypic instability, or other undesired changes may occur after transfection [1,134]. Although the proliferative characteristics of hTERT are well documented, aberrant karyotypic structures can be detected at early passages [135,136]. Perhaps the best hope for circumventing the uncertainties of immortalized cell lines is to harvest MSCs from more plentiful sources of tissue, such as umbilical cord blood or adipose tissue, which, by virtue of sheer volume at the time of harvest, can provide sufficient numbers of cells within a few passages. Notably, adipose-derived MSCs are similar to those isolated from bone marrow in terms of morphology, the success rate of isolating MSCs, expansion potential, differentiation capacity, and immunophenotype [137,138], suggesting that they may be an ideal alternative source of MSCs.

A second potentially harmful aspect of transgenic cell therapy is the possibility of insertional mutagenesis. The term *insertional mutagenesis* refers to the insertion of an otherwise non-cancerous transgene at a critical genetic locus, resulting in dysregulation of the normal mechanisms of tumor suppression, and ultimately, oncogenic consequences [139,140]. The dangers of insertional mutagenesis were highlighted in a French clinical trial for severe combined immunodeficiency that employed uncloned autologous hematopoietic stem cells transduced with a retrovirus encoding the IL2RG gene [141–143]. The exogenous nucleotide sequences were found adjacent to loci encoding *LMO2, LYL1, c-Jun, Bmi1*, and *CCND2*, upregulating the expression of these genes, which in turn transformed T cells [142,144]. Four of the 10 patients in the trial developed a T-cell acute lymphoblastic leukemia-like disease [144–148]. Such devastating outcomes suggest that in transgenic cell-based therapies, either well-characterized cell lines must be used, or robust methods for characterizing the potential insertion sites must be developed, to lower the potential for insertional mutagenesis. This potential for insertional mutagenesis during the genetic modification of MSCs will be both difficult and costly to overcome, and may very well remain one of the greatest limiting factors in the translation of MSCs as gene-delivery vehicles.

LIMITATIONS OF EVIDENCE FROM CLINICAL TRIALS

Available results from clincial trials do not represent the optimal source of evidence on the potential tumorigenic capacity of MSCs, as these trials rarely focused on parameters which are relevant for assessing the development of new cancer. Twenty four clinical trials employing MSCs have been published to date [149–162]. Most of these trials have evaluated the safety and efficacy of MSCs within the context of non-cancerous conditions, such as myocardial damage [152,157,162,163] and prevention of graft versus host disease [36,153,164–166].

There have been no reports of neither acute nor long-term adverse events thus far. This includes no reports of carcinogenesis or major adverse events from allogeneic transplants. Although these clinical trials represent the best source of evidence for the efficacy of MSCs in the treatment of mostly non-cancerous disease, initial clinical trials of novel therapies, including those using MSCs, are usually insufficient to detect the carcinogenic potential of the agent that is being tested. The longest follow-up interval in published clinical trials employing MSCs is about 3 years [149]. As carcinogenesis is often a prolonged process, more rigorous and long-term follow-up will be required to adequately detect the formation (or prolongation) of cancer in patients treated with MSCs. A second limitation of existing trials of MSCs is that they have only been conducted in very ill patients with poor prognoses, which may obscure any possible harmful effects of MSCs. Third, clinical trials to date have failed to employ imaging modalities that are capable of detecting the presence of cancer in many locations in the body. Finally, genetically-modified MSCs have rarely been used in published clinical trials [33]. Although clinical trials to date have not revealed any evidence of carcinogenesis, their reliability in determining carcinogenic potential is limited by the factors described above.

The gold standard for establishing carcinogenic potential of an agent is the triad of epidemiological studies demonstrating *association*, animal studies demonstrating *causality*, and experimental studies demonstrating *mechanism* [167]. Although considerable advances have been made with regard to the potentially carcinogeneic mechanisms of MSCs, animal studies require much more refined cancer models, and epidemiological studies will require more refined clinical methods. As longer follow up intervals and patient enrollment are achieved in clinical studies, in combination with animal tumor models that recapitulate the stromal environment of human tumors, it may one day be possible to understand more completely the risks of MSC-based therapies.

CONCLUSION: ARE MESENCHYMAL STEM CELLS SAFE TO ADMINISTER TO PATIENTS?

To summarize, the overall impression that emerges from the published literature on MSCs is that they hold tremendous promise for the treatment of cancer as well as many other diseases. These claims of therapeutic efficacy have been substantiated in large part by the few clinical trials that have been conducted to date using MSCs in the treatment of myocardial damage [152,157,162,163] and graft versus host disease [36,153,164–166]. In addition, almost all animal studies that have employed genetically-modified MSCs for the treatment of cancer (Table 2) have demonstrated therapeutic—not harmful—effects. However, the oncogenic potential of MSCs remains a poorly explored, although tremendously important, component to realizing the full clinical potential of MSCs. This review summarized how MSCs may undergo malignant transformation *in vitro* during production phases, by interactions with tumor stroma *in vivo*, or through genetic modifications with transgenes. Furthermore, the limitations of existing clinical trials, and in particular, the need for adequate follow-up protocols to screen for the presence of developing cancers, were addressed. Rigorous evaluations of the oncogenic risk of MSCs are currently needed, as well as more thorough elucidation of the molecular mechanisms that underlie their documented biological properties. Both will help to define the scope of limitations of MSC-based therapies, and will underlie the development of future techniques that, it is hoped, can overcome these limitations and facilitate the translation of MSCs to clinical use. The tremendous therapeutic potential of MSCs for the treatment of cancer [1,14,16,31,168] heightens the urgency of such studies that can both define and overcome their potential dangers.

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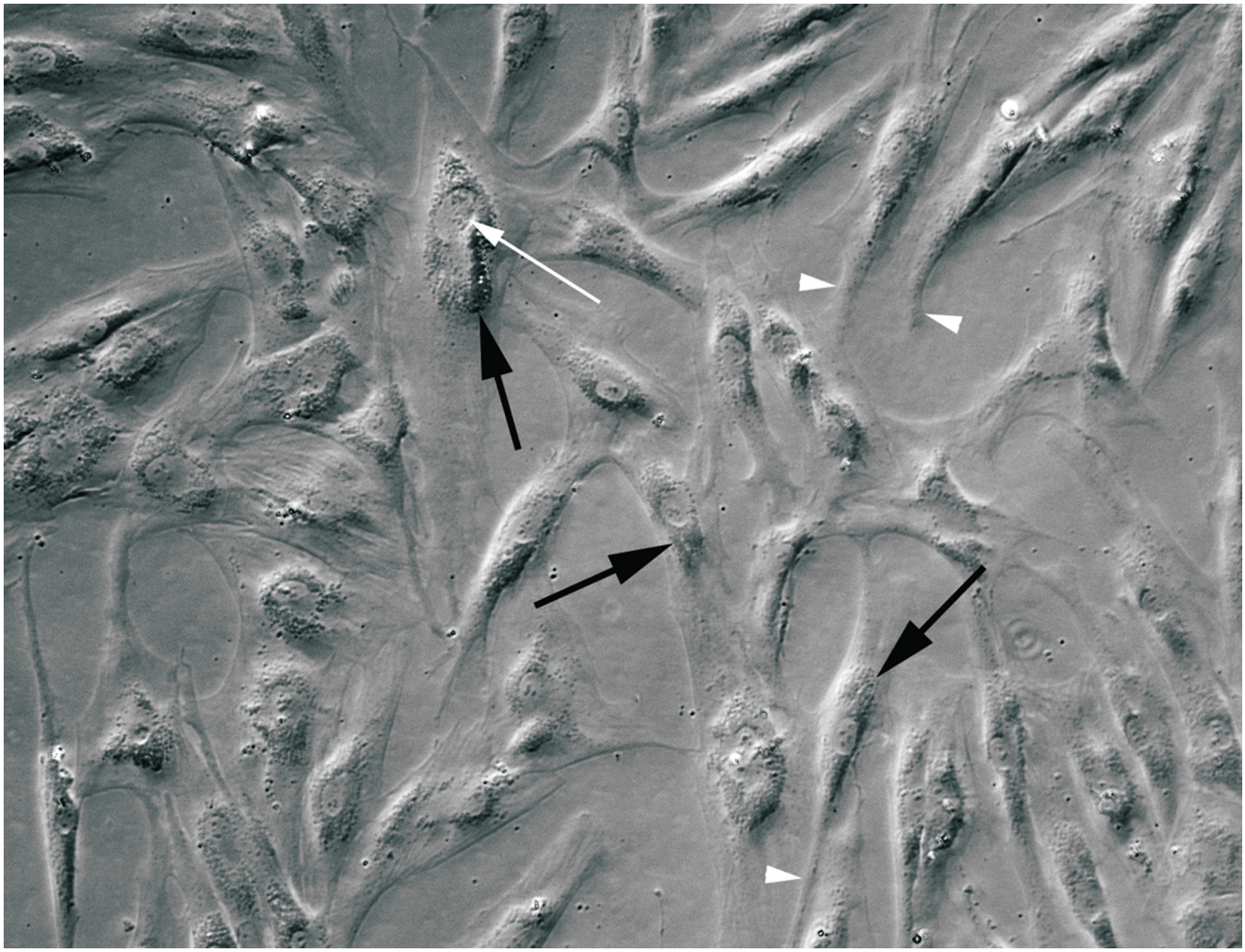
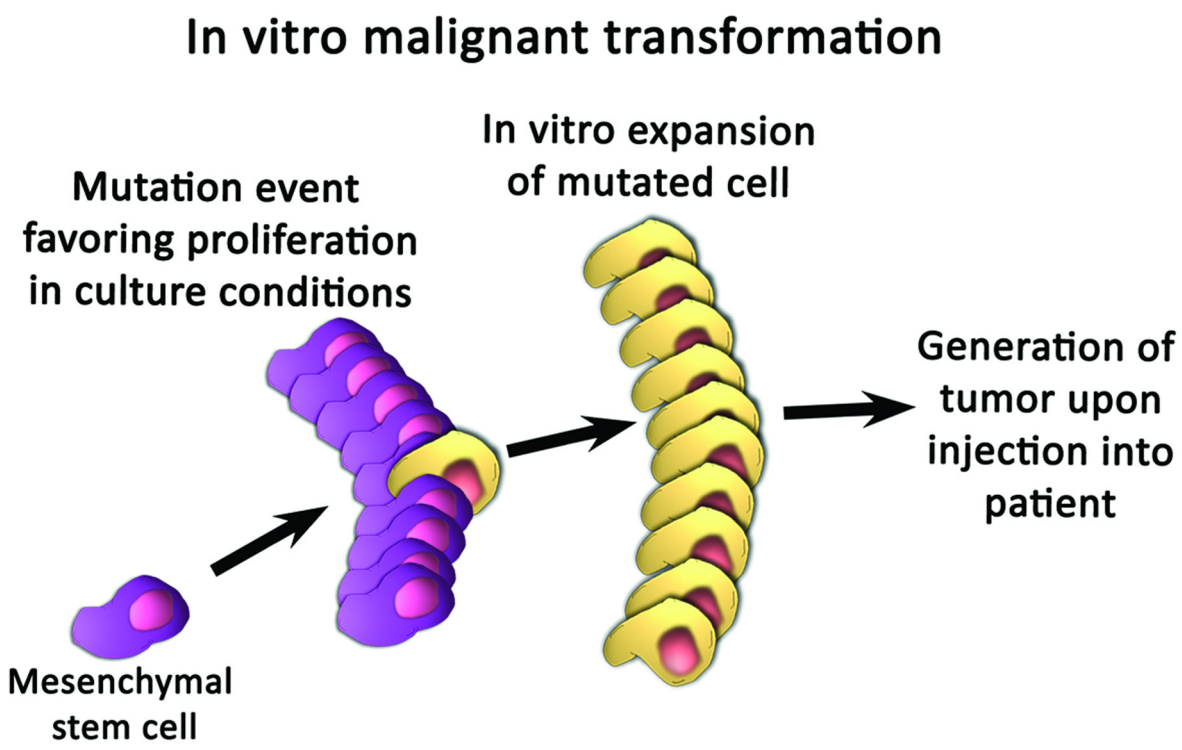


Figure 1.
Human mesenchymal stem cells at first passage display characteristic morphology: large nuclei (black arrows), prominent round nucleoli (white arrow), and long thin processes (arrowheads).



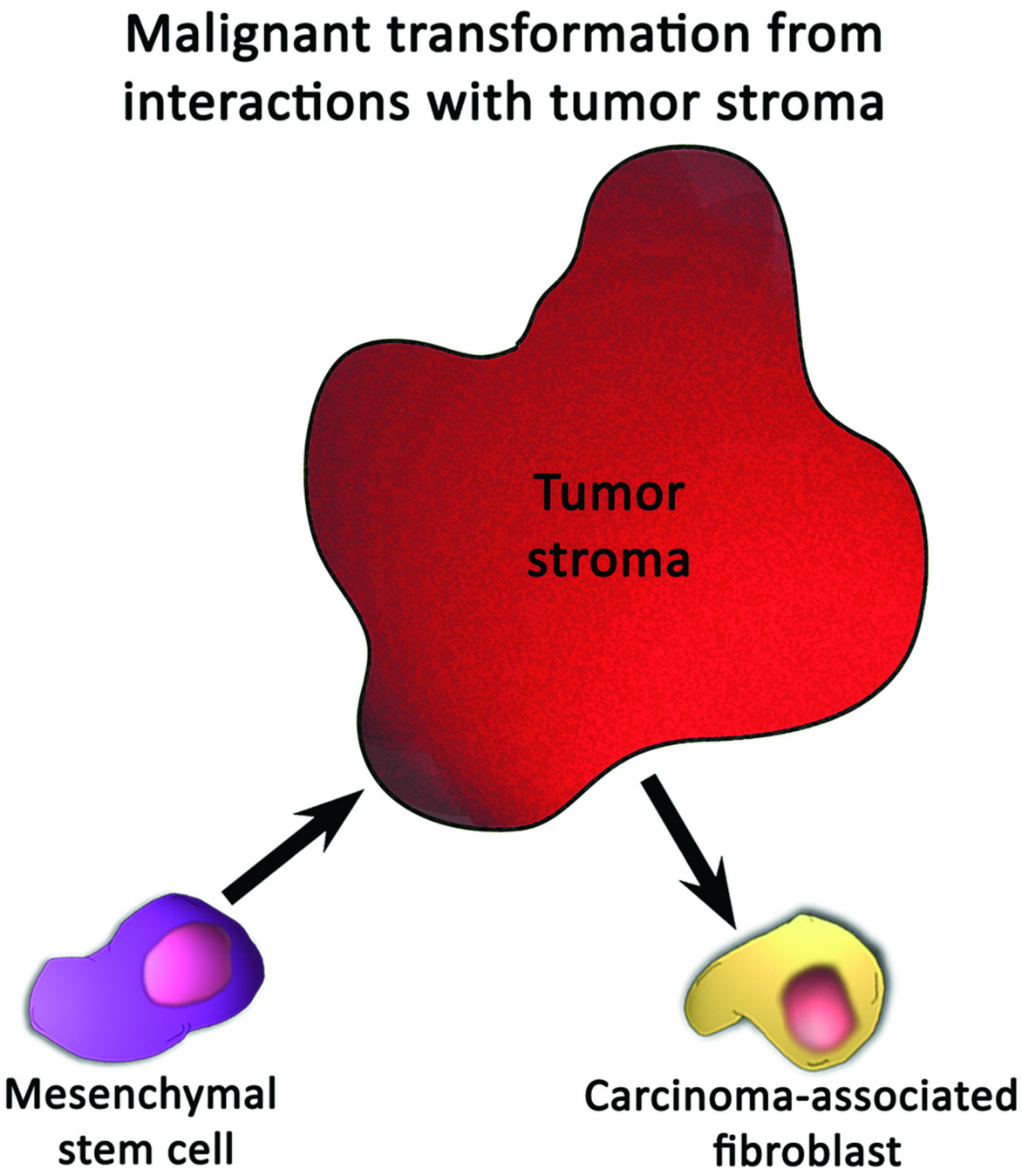
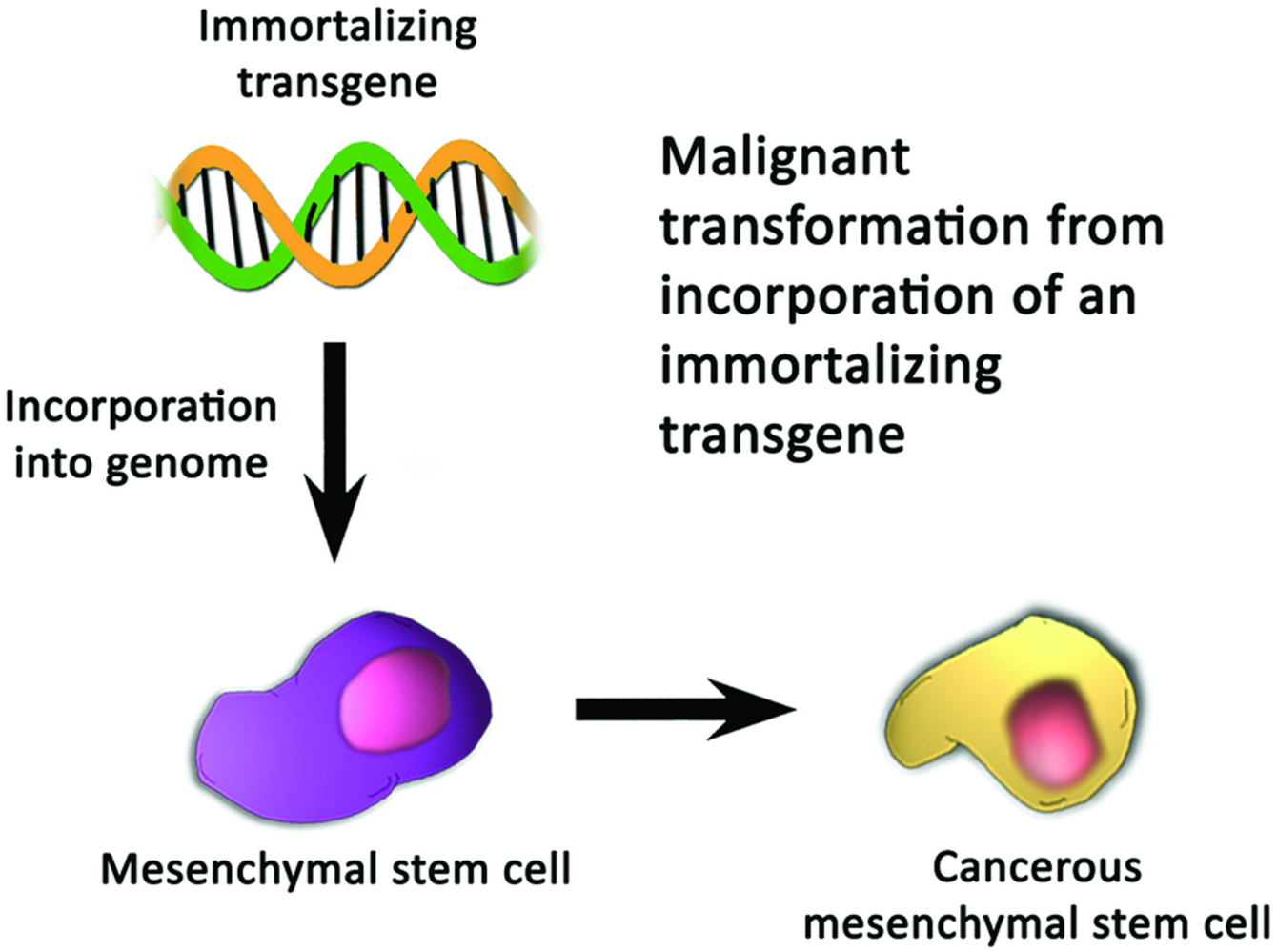


Figure 2.
Mechanisms by which mesenchymal stem cells may undergo carcinogenic transformation. **(A)** Transformation may occur by the outgrowth of a sub-population of cells that proliferates more favorably in culture conditions. **(B)** Interactions between tumor stroma and mesenchymal stem cells may cause mesenchymal stem cells to differentiate into carcinoma-associated fibroblasts.



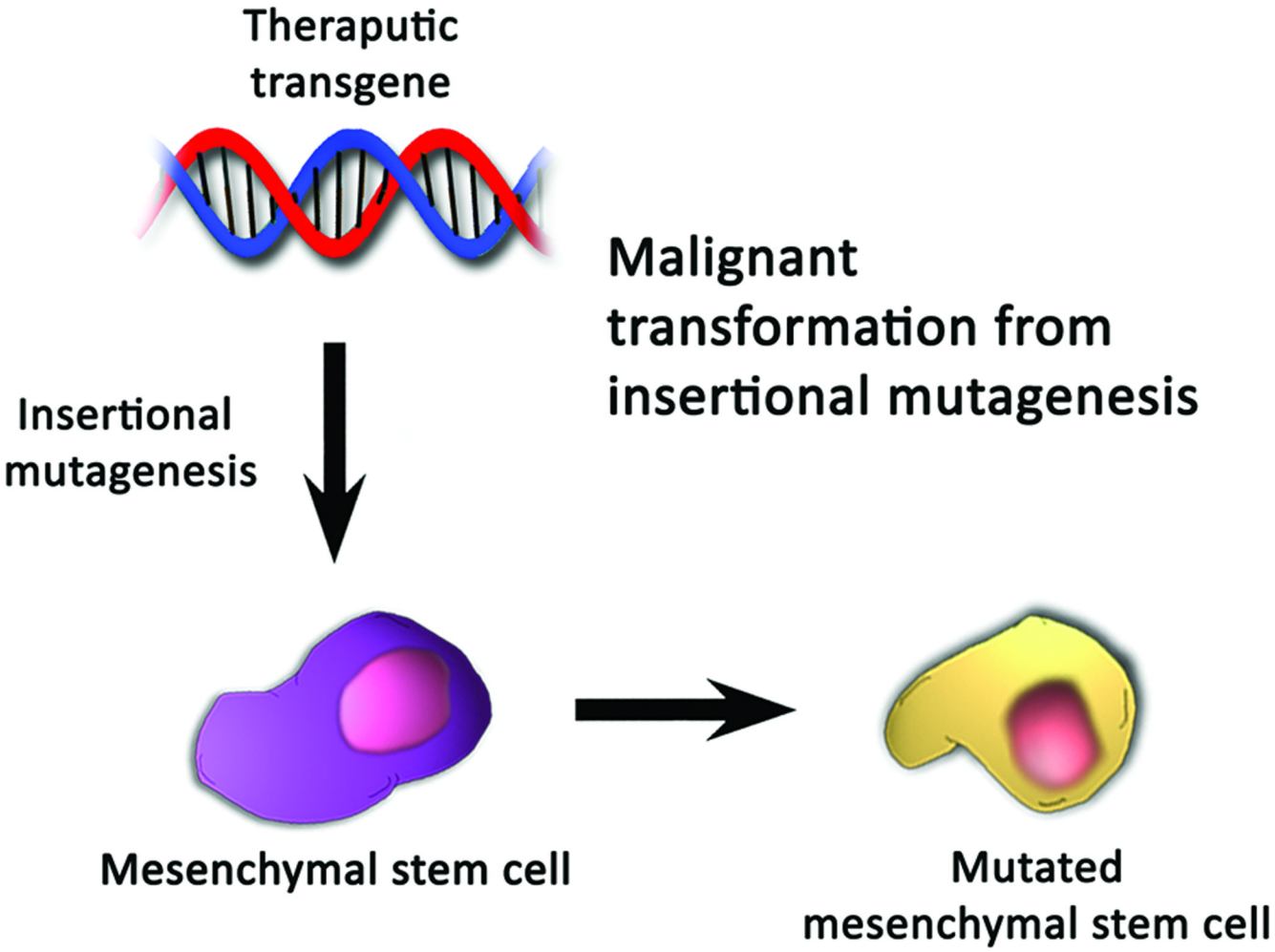


Figure 3.
Mechanisms by which mesenchymal stem cells may become oncogenic after incorporation of a transgene. **(A)** Incorporation of an immortalizing transgene may result in the loss of normal cell-cycle checkpoints, karyotypic instability, or other changes which cause uninhibited proliferation of cells. **(B)** Insertional mutagenesis may disrupt a critical regulatory locus, resulting in dysfunction of the normal mechanisms of tumor suppression.

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Table 1

***In vivo* studies of unmodified mesenchymal stem cell migration**

Additional studies on the migration of other cell types, such as neural stem cells, are reviewed elsewhere [1].

CITATION	HOST SPECIES	PRECONDITIONING OR PRE-EXISTING CONDITION	IMPLANTED CANCER CELL DESCRIPTION	IMPLANTED MSC DESCRIPTION	MSC INJECTION ROUTE	MAIN RESULTS / MSC DISTRIBUTION PATTERNS
Pereira *et al.* 1998 [30]	Mouse	Osteogenesis imperfecta animal model; lethal or sublethal irradiation	None	Murine BM-MSC	IV	MSCs migrated primarily bone marrow, cartilage, lung, but also in spleen, brain, skin.
Maestroni *et al.* 1999 [169]	Mouse	None	Lewis lung carcinoma, B16 melanoma	Bone marrow cells, unspecified source	IM	Inhibition of tumor growth and number of metastases.
Koç *et al*. 2000 [170]	Human	Patients underwent myeloablative therapy and then received hematopoietic stem cell graft	Pre-existing breast cancer	Human BM-MSC	IV	MSCs detected in blood up to 1 hour after infusion. Revived neutrophil and platelet count in 8 days.
Gao *et al*. 2001 [29]	Rat	Either sodium Nitroprusside administration or none.	None	Rat BM-MSC	IA, IV, IP	Migration to lungs, liver, bone marrow. With nitroprusside, high levels in liver and bone marrow.
Le Blanc *et al.* 2004 [36]	Human	Patient had grade IV acute graft-versus-host disease	None	Human BM-MSC	IV	Migration to colon and lymph node. Recovery after 1 year.
Houghton *et al.* 2004 [37]	Mouse	Chronic gastrointestinal inflammation from *Helicobacter* infection	None	Endogenous MSCs were studied	N/A	Repopulation of the stomach with MSCs, which progress through metaplasia, dysplasia, and cancer.
Khakoo *et al.* 2006 [171]	ID Mouse	None	Kaposi's sarcoma animal model	Human BM-MSCs	IV	Inhibition of tumor growth.
Zhu *et al*. 2006 [46]	ID Mouse	None	F6 and SW480 colon cancer	Fetal and adult human BM-MSC	SQ	Higher tumor incidence in MSC-treated groups, with elevated proliferation, angiogenesis, and metastatic ability of cancer cells.
Ramasamy *et al.* 2007[34]	NOD/ SCID Mouse	None	BV173 chronic myeloid leukemia	Human BM-MSC co-injected with tumor cells	SQ	Migration to bone marrow. Higher incidence of tumor formation in MSC-treated groups. Lower rates of cancer cell apoptosis.
Karnoub *et al.* 2007 [43]	NOD/ SCID Mouse	None	MCF7/Ras, MDA-MB-231, MDA-MB-435, and HMLER breast cancer	Human BM-MSC co-injected with tumor cells	SQ	Enhanced motility, invasion, and metastasis of cancer cells.

Abbreviations: ***AMSC*** = Adipose tissue-derived mesenchymal stem cell; ***BM-MSC*** = Bone marrow-derived mesenchymal stem cell; ***CT*** = Contralateral to tumor; ***IA*** = Intra-arterial; ***ID*** = Immunodeficient; ***IFN*** = Interferon; ***IL*** = Interleukin; ***IM*** = Intramuscular; ***IP*** = Intraperitoneal; ***IT*** = Intratumoral; ***IV*** = Intravenous; ***MFP*** = Mammary fat pad; ***MSC*** = Mesenchymal stem cell; ***neo^R*** = Neomycin phosphotransferase gene; ***PT*** = Peritumoral; ***SCID*** = Severe combined immunodeficient; ***SQ*** = Subcutaneous; ***TRAIL*** = Tumor necrosis factor-related, apoptosis-inducing ligand; ***tsFlk-1*** = Truncated soluble vascular endothelial growth factor receptor gene.

Table 2

***In vivo* studies of mesenchymal stem cells genetically modified by incorporation of a transgene**

Abbreviations are the same as for Table 1.

CITATION	HOST SPECIES	PRECONDITIONING OR PRE-EXISTING CONDITION	IMPLANTED CANCER CELL DESCRIPTION	IMPLANTED MSC DESCRIPTION	MSC TRANSGENE	MSC INJECTION ROUTE	MAIN RESULTS / MSC DISTRIBUTION PATTERNS
Studeny *et al.* 2002 [172]	ID Mouse	None	A375SM melanoma lung metastasis	Human BM-MSC	IFN β	SQ, IV	Extended survival in tumor implanted animals treated with IV IFN β-secreting MSCs.
Horwitz *et al.* 2002 [33]	Human	Prior bone marrow transplantation	None	Human BM-MSC	neo^R	IV	Migration to bone marrow, bone, skin. Accelerated growth velocity 6 months following MSC transplant.
Devine *et al.* 2003 [28]	Baboon	Lethal irradiation + hematopoietic stem cells; or no conditioning	None	Baboon BM-MSC	GFP	IV	Migration to gastrointestinal tract, kidney, lung, liver, thymus, and skin. Nonconditioned animal had less abundant engraftment.
Studeny *et al.* 2004 [173]	SCID mouse	None	MDA231 breast cancer, A375SM pulmonary metastasis	Human BM-MSC	IFN β	IV	Extended survival.
Nakamura *et al.* 2004 [174]	Rat	None	9L glioma	Rat BM-MSC	Il-2	CT, IT	Migration toward tumor. Extended survival, tumor volume reduction.
Nakamizo *et al.* 2005 [15]	ID Mouse	None	U87, U251, LN229 glioma implantation	Human BM-MSC	IFN β	IA, IT	Migration toward tumor. Extended survival in U87 implanted animals treated with IFN β-secreting MSCs.
Zappia *et al.* 2005 [35]	Mouse	Autoimmune encephalomyelitis animal model	None	Murine BM-MSC	Enhanced GFP	IV	Migration to lymphoid organs, subarachnoid space. Decreased inflammatory infiltrates and demyelination in mice treated with MSCs.
Elzaouk *et al.* 2006 [175]	Mouse	None	B16F10 local melanoma, and melanoma lung metastasis	Human MSCs, unspecified source	Rat IL-12	IP, IM, IT	Decreased tumor volume and metastasis seeding.
Komarova *et al.* 2006 [176]	SCID Mouse	None	SKOV 3 ovarian carcinoma	Human BM-MSC	Ad5/3	IP	Enhanced survival.
Djouad *et al.* 2006 [44]	Mouse	None	Renca adenocarcinoma pulmonary metastasis, B16 melanoma, animal model	Murine MSC	Luciferase	IV	No effect on tumor volume, but earlier onset of tumors in MSC treated groups.

CITATION	HOST SPECIES	PRECONDITIONING OR PRE-EXISTING CONDITION	IMPLANTED CANCER CELL DESCRIPTION	IMPLANTED MSC DESCRIPTION	MSC TRANSGENE	MSC INJECTION ROUTE	MAIN RESULTS / MSC DISTRIBUTION PATTERNS
Kyriakou *et al.* 2006 [177]	ID Mouse	None	Raji Burkitt lymphoma	Human BM-MSC	tsFlk-1	SQ	Inhibition of tumor growth
Kucerova *et al.* 2007 [32]	ID Mouse	None	HT-29 colon adenocarcinoma	Human AMSC	Cytosine deaminase	SQ, IV	Migration to lungs and liver, but cleared at 20 days. Inhibition of tumor growth.
Miletic *et al.* 2007 [178]	Rat	None	9L glioma	Rat BM-MSC	Thymidine kinase, eGFP	IT	Extended survival.
Hong *et al.* 2009 [16]	Mouse	None	Ast11.9-2 glioma	Murine BM-MSC	Murine IL-12	PT	Migration toward tumor. Extended survival.
Duan *et al*. 2009 [31]	Mouse	None	TC71 Ewing sarcoma tumors	Murine BM-MSC	IL-12	IV	Migration toward tumor, lung, liver, and spleen. Inhibition of tumor growth.
Yang *et al*. 2009 [14]	ID Mouse	None	U87 glioma	Human BM-MSC	TRAIL	IV	Migration to tumor and kidney, with lower levels in liver, lung, spleen. Extended survival in MSC-TRAIL groups.
Sasportas *et al.* 2009 [168]	ID Mouse	None	GBM8 CD133+ human glioma	Human BM-MSC	TRAIL, luciferase	PT	Migration toward tumor. Extended survival in MSC-TRAIL groups.