MSCs derived from iPSCs with a modified protocol are tumor-tropic but have much less potential to promote tumors than bone marrow MSCs

Qingguo Zhao, Carl A. Gregory, Ryang Hwa Lee, Roxanne L. Reger, Lizheng Qin, Bo Hai, Min Sung Park, Nara Yoon, Bret Clough, Eoin McNeill, Darwin J. Prockop, and Fei Liu

Institute for Regenerative Medicine, Texas A&M Health Science Center College of Medicine at Scott & White, Temple, TX 76502

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Mesenchymal stem or stromal cells (MSCs) have many potential therapeutic applications including therapies for cancers and tissue damages caused by cancers or radical cancer treatments. However, tissue-derived MSCs such as bone marrow MSCs (BM-MSCs) may promote cancer progression and have considerable donor variations and limited expandability. These issues hinder the potential applications of MSCs, especially those in cancer patients. To circumvent these issues, we derived MSCs from transgene-free human induced pluripotent stem cells (iPSCs) efficiently with a modified protocol that eliminated the need of flow cytometric sorting. Our iPSC-derived MSCs were readily expandable, but still underwent senescence after prolonged culture and did not form teratomas. These iPSC-derived MSCs homed to cancers with efficiencies similar to BM-MSCs but were much less prone than BM-MSCs to promote the epithelial-mesenchymal transition, invasion, stemness, and growth of cancer cells. The observations were probably explained by the much lower expression of receptors for interleukin-1 and TGFβ, downstream protumor factors, and hyaluronan and its cofactor TSG6, which all contribute to the protumor effects of BM-MSCs. The data suggest that iPSC-derived MSCs prepared with the modified protocol are a safer and better alternative to BM-MSCs for therapeutic applications in cancer patients. The protocol is scalable and can be used to prepare the large number of cells required for “off-the-shelf” therapies and bioengineering applications.

mesenchymal stem cells | cancer | iPS cells | tumor tropism | protumor effects

The use of mesenchymal stromal or stem cells (MSCs) in cancer patients or cancer survivors is a promising strategy to improve treatment of advanced cancer (1) and to repair tissues damaged by cancers or by radical cancer therapies (2). Based on the unique homing capability of tissue-derived MSCs to stroma of various primary and metastatic cancers (3–6), MSCs have the potential to treat or even eliminate various cancers by delivering various anticancer agents (7–9). Because of their potential for differentiation (10, 11) and production of immunomodulatory, angiogenic, anti-apoptotic, anti-scarring, and prosurvival factors (12), MSCs have shown promising regeneration potential after radical cancer treatment in animal models, such as soft tissue reconstruction after disfiguring surgeries for head, neck, or breast cancers (13) and salivary gland regeneration for head and neck cancer patients treated with radiotherapy (14, 15). As one example, the combination of osteogenic potential and targeted delivery of anticancer agents make MSCs a promising option to treat tumor-induced osteolysis (16, 17). However, exogenous tissue-derived MSCs, including those from bone marrow, adipose tissues, and umbilical cord, have all shown a tendency to promote rather than inhibit cancers in many circumstances (18–23). Also, endogenous MSCs are a major source of reactive stromal cells that promote growth and metastasis of cancers (4, 24).

Moreover, MSCs have a limited proliferation potential and lose some of their important biological functions as they are expanded (25). Therefore, it is difficult to prepare large banks of the cells with uniform biological activities and/or transgene expression required for experiments in large animals and for potential clinical therapies. Another problem is that MSCs are being prepared with a variety of protocols in different laboratories from different donors. As a result, standardization of the cells has been extremely difficult and the data presented in different publications are difficult to compare. Hence large banks of reference cells are needed to advance the MSC research (26).

To address the limitations of expandability and standardization, we derived MSCs from induced pluripotent stem cells (iPSCs) with a modified protocol that can be expanded to provide large cell banks from a single cell clone. The protocol produces highly enriched MSC-like cells from iPSCs with high efficiency. The iPSC-derived MSCs (iPSC-MSCs) express the classical surface markers of MSCs, are capable of multilineage mesodermal differentiation and cancer homing, and can be expanded extensively, but do not preserve the pluripotency of iPSCs. Surprisingly, iPSC-MSCs do not promote epithelial–mesenchymal transition (EMT), invasion, and stemness of cancer cells as is seen with bone marrow-derived MSCs (BM-MSCs). Consistent with these observations, the iPSC-MSCs express much lower levels than BM-MSCs of protumor factors including interleukin-6, prostaglandin E2, SDF1, and hyaluronan before and after exposure to tumor microenvironment. Our data indicated that iPSC-MSCs are a safe alternative to BM-MSCs for

Significance

Mesenchymal stem or stromal cells (MSCs) offer great promise as potential therapies for cancers and other diseases. However, applications of MSCs for cancer therapy are hindered by their protumor potential under certain conditions, considerable donor variations, and limited expandability. Here we report generation of induced pluripotent stem cells (iPSC)-derived MSCs with same tumor homing capacity but much less protumor potential using a modified protocol with high derivation efficiency. Starting from iPSCs with almost unlimited expandability, the protocol can be readily scaled up to provide huge amounts of MSCs with uniform biological properties for multicenter evaluation, large animal experiments, and potential clinical trial. Moreover, therapeutic transgenes can be inserted into safe-harbor loci of iPSCs before derivation of MSCs to eliminate insertional mutation and guarantee stable expression of transgenes during prolonged expansion.


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1To whom correspondence may be addressed. Email: prockop@medicine.tamhsc.edu or flu@medicine.tamhsc.edu.

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cancer therapy and other applications with better expandability and potential for genetic engineering.

Results
Derivation of MSC-Like Cells from Human iPS Cells. Sánchez et al. (27) reported that inhibition of SMAD-2/3 signaling promoted derivation of MSCs from human embryonic stem cells (ESCs) but not from human iPSCs. To derive MSCs efficiently from human iPSCs, we modified their method by using chemically defined mTeSR1 medium (28) supplemented with the SMAD-2/3 inhibitor (SB-431542) and an atmosphere of 7.5% CO2 (29) to culture colonies of cells on Matrigel-coated plates. The cells were passaged at 80–90% confluency by lifting with Dispase. After about 25 d, most cells became larger with increased cytoplasm, and cells at the edge of the cell cluster became spindle-shaped, suggesting spontaneous differentiation (Fig. 1A). We then digested the cultures with trypsin to generate suspensions of single cells and transferred them to standard tissue culture plates. The cells were incubated in ESC–MSC medium (30) containing SB-431542, lifted at 80–90% confluency by trypsin every 3 d, diluted 1:3, and passaged repeatedly under the same conditions (4431). During repeated passaging by trypsinization, more and more adherent cells gradually showed spindle-like morphology and appeared in whorls similar to MSCs and fibroblasts (Fig. 1A). After 45 d, quantitative RT-PCR (qRT-PCR) analysis indicated there was a marked decrease in the expression of the pluripotent genes Nanog and Oct4, the neuroectoderm marker Ecad, and the endoderm marker Foxa2 in the adherent cells. In contrast, there was a marked increase in the expression of the mesodermal marker CD140A/Pdgfra (Fig. 1B, n = 0.001). Flow cytometric analysis indicated that, similar to BM-MSCs, positive MSC markers were expressed by the vast majority of adherent cells (>99.6% for CD73, CD105, and CD166, and >88.4% for CD44 and CD90), whereas negative MSC markers including HLA-DR, CD11b, CD24, CD34, and CD45 were expressed by a very small fraction of these cells (<4.1%, Fig. 1C). When incubated in standard osteogenic media, the adherent cells were remarkably osteogenic, generating a fully differentiated monolayer of mineralizing MSCs within 10 d, about half of the time required for BM-MSCs (Fig. 1D). The adherent cells also generated cartilage in micromass cultures in the presence of both BMP2 and TGFβ (Fig. 1D). In contrast, when the cells were exposed to routine adipogenic conditions for a standard duration of 20 d, they were modestly responsive compared with BM-MSCs (Fig. 1D). qRT-PCR analysis indicated that in iPSC-MSCs the up-regulation of most markers for osteoblasts or chondrocytes after corresponding differentiation was significantly higher, whereas the up-regulation of most adipogenic markers was significantly lower compared with BM-MSCs (Fig. 1E). Because the adherent cells met the standard criteria of MSCs (32), they were subsequently referred to as iPSC-MSCs and designated as passage 0. As expected, telomerase activity in iPSC-MSCs was much higher than that in BM-MSCs (passage 4) but much lower than that in parent iPSCs (passage 39) (Fig. 1F). Consequently, the colony-forming unit–fibroblast (CFU–F)-forming efficiency of iPSC-MSCs was also much higher than that of BM-MSCs at passages 4 and 12 (Fig. 1G), indicating better expandability of iPSC-MSCs compared with BM-MSCs. The average population doubling time from passages 3–15 for iPSC-MSCs was significantly shorter than that of BM-MSCs (25.28 ± 2.92 vs. 33.91 ± 5.03 h, mean ± SD, n = 3, P < 0.05), indicating that iPSC-MSCs propagate more rapidly than BM-MSCs. However, iPSC-MSCs were not immortal in culture. They underwent senescence and could not be expanded beyond 17 passages (64 population doublings), similar to BM-MSCs cultured under the same conditions that underwent senescence after 16 passages (48 population doublings) (Fig. 1H).

Cytogenetic analysis indicated that iPSC-MSCs at passage 7 had a normal karyotype (Fig. S1), and no teratoma formation was observed in non-obese diabetic (NOD)/SCID mice inoculated with iPSC-MSCs after 4 mo. The data indicated therefore that we have developed an efficient and safe protocol to derive MSCs from human iPSCs.

Human iPSC-MSCs Were Capable of Homing to Tumors. MSCs from various tissues have a unique tumor-homing capacity that enables them to serve as vehicles for gene therapy of advanced cancers. The tumor tropism of these MSCs is mediated by multiple chemokine receptors such as CXCR4 and CXCR6 (4–6), CD44 (33), and VEGFR1 (3) and integrins such as ITGA6 and ITGB1 (34, 35). The expression of VEGFR1 was dramatically higher in iPSC-MSCs than in BM-MSCs, whereas the expression of other homing-related genes was comparable between iPSC-MSCs and BM-MSCs (Fig. 2A). In vitro transwell migration experiments showed that there was significantly increased migration of BM-MSCs to MDA-MB-231 cells, a line of triple-negative human breast cancer cells, compared with control human embryonic kidney 293T cells.
Similar results were reported previously (8). The migration of iPSC-MSCs to MDA-MB231 cells was similarly significantly increased compared with that of 293T cells or medium alone (Fig. 2B, P < 0.01), and was comparable to that of BM-MSCs to MDA-MB231 breast cancer cells. After tumor establishment, BM-MSCs or iPSC-MSCs transduced with CMV-copGFP lentivirus were injected into tumor-bearing mice intravenously. To quantify the homing of MSCs to cancer, we developed individual standard curves of CMV qPCR for BM-MSCs or iPSC-MSCs carrying CMV-copGFP by adding varying amounts of genomic DNA (gDNA) from corresponding cells to gDNAs of LoVo or MDA-MB-231 tumor tissues from mice without infusion of MSCs (Fig. 2C, R² > 0.97). Sixteen hours after MSC infusion, qPCR of CMV promoter sequence indicated that infused BM-MSCs and iPSC-MSCs homed to LoVo or MDA-MB-231 tumors with comparable efficiencies (Fig. 2D and E, P > 0.05). Consistent with these observations, GFP+ cells were found in sections of LoVo or MDA-MB-231 tumor samples from mice infused with BM-MSCs or iPSC-MSCs carrying CMV-copGFP (Fig. 2F). Taken together, these data indicated that iPSC-MSCs are capable of homing to cancer similarly to BM-MSCs.

**iPSC-MSCs Had Less Potential than BM-MSCs to Promote Epithelial-Mesenchymal Transition, Invasion, and Cancer Stem Cell Expansion**

Interactions between carcinoma cells and MSCs promote metastasis and/or expansion of the cancer stem cells by enhancing EMT (19). We first compared the potential of iPSC-MSCs and BM-MSCs to enhance EMT of cocultured cancer cells. Cancer cells were transduced with CMV-copGFP lentiviruses, cocultured with MSCs, and then sorted by FACS. In LoVo cancer cells, 12 h of cocultures with BM-MSCs significantly decreased the expression of the epithelial marker E-cadherin (Ecad) and significantly increased expression of mesenchymal makers fibronectin1 (FN1), N-cadherin (NCAD), vimentin (VIM), and metalloprotease 2 (MMP2) as well as the pro-EMT factors ZEB1, ZEB2, and TWIST1 (Fig. 3A). In contrast, coculture with iPSC-MSCs did not significantly decrease expression of Ecad and had either no significant effects on or produced much smaller increases of the expression of these mesenchymal markers or pro-EMT genes in LoVo cells (Fig. 3A). The results demonstrated therefore that the iPSC-MSCs had less potential to promote EMT than BM-MSCs. To determine whether the iPSC-MSCs promoted invasion of cancer cells, an invasion assay using collagen IV-coated Boyden chambers was used. After coculture with BM-MSCs for 3 d, invasion of LoVo, HCC1806, and MCF7 human cancer cells was significantly increased (Fig. 3B, P < 0.05). In contrast, there was no significant increase after coculture with iPSC-MSCs (Fig. 3B, P > 0.05). To test whether the iPSC-MSCs promoted expansion of cancer stem cells (CSCs), we examined the aldehyde dehydrogenase (ALDH) population in LoVo colorectal cancer cells that are enriched for CSCs (19). After coculture with BM-MSCs for 5 d, there was significant expansion of the ALDH+ cells (Fig. 3C). Coculture with iPSC-MSCs had no significant effect. Similar effects of iPSC-MSCs on expression of EMT-related genes and ALDH+ population were observed in HCC1806, another line of human triple-negative breast cancer cells (Fig. S2). Consistent results were obtained with another assay for cancer stem cells: the mammosphere-forming capacity that is characteristic of breast cancer stem cells in cultures of MCF7 and HCC1806 cells (36). After coculture for 3 d, mammosphere formation of FACS-isolated breast cancer cells was significantly increased by BM-MSCs but not by iPSC-MSCs (Fig. 3D). Coinoculation with BM-MSCs remarkably increased the tumor-initiating ability and the tumor growth of multiple types of cancer cells including HCC1806 (19); however, we found that the tumor-initiating ability and the weights of tumors of HCC1806 cells coinoculated with iPSC-MSCs were significantly lower than those coinoculated with BM-MSCs when either 5 × 10⁴ or 5 × 10⁵ HCC1806 cancer cells were injected (P < 0.05 for tumor weight, Fig. 3E). Collectively, these data indicated that our iPSC-MSCs are less prone to support the growth and invasion of cancers than BM-MSCs.

**Activity of IL1R-PGE2-IL6 Pathway Was Marginal in iPSC-MSCs.** For cancer cells such as LoVo and HCC1806 that express a high level of interleukin-1 (IL1), the protumor effect of MSCs is mediated mainly by the IL1 receptor (IL1R)/prostaglandin E2 (PGE2) pathway (19). Our iPSC-MSCs compared with BM-MSCs expressed much lower levels of mRNA for IL1R type 1 (IL1R1), the signal transducer of IL1 pathway (37), and prostaglandin E synthase (PTGES/mPGES1). The much lower levels of expression of these two genes were not significantly affected by treatment with 0.25 ng/mL IL1β, tumor-conditioned medium (TCM) from LoVo cells or coculture with LoVo cells (Fig. 4A). The basal level of expression of cyclooxygenase-2 (Cox2/Ptgs2), another key PGE2 synthase, was about the same in BM-MSCs and iPSC-MSCs cultured in αMEM, but there was less up-regulation of Cox2 in iPSC-MSCs than in BM-MSCs after treatment with IL1 or LoVo TCM or coculture with LoVo cells (Fig. 4A, P < 0.05). As expected from these observations, the level of PGE2 in the medium of iPSC-MSCs was lower than that in BM-MSCs after treatment with IL1, LoVo TCM, or LoVo cells (Fig. 4B, P < 0.01). As a consequence, the expression of Interleukin-6 (IL6), a major protumor factor regulated by both IL1 and PGE2, was much lower in...
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Signaling is also essential for receptor type 1 (\( < 0.05 \), Fig. 5) and dominant (\( < 0.05 \), Fig. 5) (TSG6). Therefore, the results indicated that 0.05, Fig. 5) Signaling and Production of Related Protumor Factors Was Less One essential mechanism of the protumor effects mRNA in -MSCs than in BM-MSCs when \( < \), Fig. 5) and to promote the growth and invasion of IL1-expressing cancer cells. These data indicate that decreased TGFβ signaling also contributes to the lack of significant protumor effects in iPSC-MSCs.

iPSC-MSCs Compared with BM-MSCs Produced Less Hyaluronan and TSG6 and Did Not Up-Regulate Lysyl Oxidase in Cocultures with Cancer Cells. One essential mechanism of the protumor effects of BM-MSCs is the up-regulation of lysyl oxidase (LOX) in adjacent cancer cells by triggering the CD44-signaling pathway with hyaluronan (HA) to promote EMT and metastasis (42). Tumor necrosis factor \( \alpha \)-induced protein 6 (TSG6), a secreted protein highly expressed by BM-MSCs (43), enhances or induces the binding of HA to cell-surface CD44 (44). In iPSC-MSCs, the expression of TSG6 and dominant IL6, and SDF1 (40), was dramatically lower than that in BM-MSCs with or without coculture with LoVo cancer cells (Fig. 6, \( P < 0.01 \)). Consistent with this observation, the amount of HA secreted into medium by iPSC-MSCs was significantly lower than by BM-MSCs at passages 5 and 15 (Fig. 6B, \( P < 0.01 \)). As expected, in cocultures with HCC1806 or LoVo cancer cells, iPSC-MSCs were less effective than BM-MSCs in up-regulating the LOX mRNA in cancer cells (Fig. 6C, \( P < 0.01 \)). Therefore, the results indicated that decreased up-regulation of LOX contributes to the lack of significant pro-EMT and proinvasion effects of iPSC-MSCs.

Discussion

The differentiation of human iPScs to MSCs has been reported to be much less efficient than the differentiation of ESCs to MSCs (20 vs. 40% CD73\(^+\)) (27). Also, flow cytometric sorting is generally necessary to isolate iPSC-derived MSCs, a procedure that is expensive, technically challenging, and may cause damage to cells. We

Fig. 3. The effects of iPSC-MSC on EMT, invasion, and cancer stem cells of cocultured cancer cells. (A–D) After coculture with GFP-labeled BM-MSCs or iPSC-MSCs, cancer cells were isolated by FACS and subjected to (A) qRT-PCR analysis of genes related to EMT and invasion. (B) Invasion assay with collagen IV-coated Boyden chambers. (C) Representative flow cytometry analysis of ALDH\(^+\) population and the percentage of ALDH\(^+\) cells (mean \( \pm \) SEM of three independent tests). (D) Mammosphere-forming assay of breast cancer cells. (E) Weights of tumors derived from HCC1806 cells injected into SCID mice with BM- or iPSC-MSCs at 6 wk.

Fig. 4. ILR-PGE2-IL6 pathway in iPSC-MSCs. (A) qRT-PCR analysis of genes of the ILR-PGE2-IL6 pathway in MSCs cultured with \( \alpha \)MEM, IL1, LoVo conditioned medium (TCM), or cells for 3 d. (B) ELISA of PGE2 in 3-d medium of MSCs cultured under above conditions.
modified the differentiation protocol by initially inhibiting Smad2/3 signaling in iPSCs cultured with chemically defined mTeSR1 medium (28) and then passaging the cells by trypsinization (31) repeatedly (30) in 7.5% CO₂ (29), conditions that were previously reported to improve the differentiation toward MSCs. During the repeated passaging by trypsinization, we used standard tissue culture plastic dishes instead of gelatin-coated plates. The early introduction of the tissue culture plastic dishes probably accelerated selection for MSC-like cells because the dishes are pretreated under proprietary conditions to increase oxygenated derivatives on the surface of the plastic and thereby make them more hydrophilic and increase the adherence of vertebrate fibroblasts and similar cells (45). The selection by adherence on the treated plastic also met one of the minimal defining criteria for human MSCs (32). This modified protocol achieved highly efficient enrichment of iPSC-MSCs (>99.6% were positive for CD73, CD105, and CD166) and eliminated the need of flow cytometric sorting. The iPSC-MSCs expanded more rapidly and to a greater extent than BM-MSCs, but still eventually underwent senescence similar to BM-MSCs. Therefore, they were less likely to cause tumors or malignancies in patients than cells that are immortal in culture (46). Also, the iPSC-MSCs did not form teratomas in mice.

The immuno-suppressive, anti-inflammatory, and differentiation properties of MSCs derived from ESCs or iPSCs have been examined by several laboratories (27, 47). However, no analysis of the tumor-homing and anti-tumor properties of ESC- or iPSC-derived MSCs has been reported. We found that our iPSC–MSCs can home to tumors with the same efficiency as BM-MSCs, but do not promote EMT, invasion, or the stemness of cancer cells as BM-MSCs do. BM-MSCs and cancer cells interact through multiple mechanisms, and the molecular profiles but not the tissue of origin of cancer cells appear to determine their interaction with MSCs. For cancer cells producing a high level of IL1 such as in LoVo colon cancer cells and HCC1806 breast cancer cells, BM-MSCs are activated by tumor-derived IL1 to produce protumor factors such as PGE2 and IL6 to promote cancer progression (19). For cancer cells producing a low level of IL1, such as MDA-MB-231 (widely metastatic) and MCF7 (noninvasive) breast cancer cells, (i) hyaluronan produced by BM-MSCs activates the CD44 pathway in cancers to induce LOX expression and promote the EMT and invasion of cancer cells (42); and (ii) TGFβ produced by cancer cells or tumor stromal cells promotes expression of protumor factors such as IL6 and SDF1 by BM-MSCs (4, 38). Intriguingly, the expression of multiple genes related to these three pathways, including receptors for IL1, TGFβ, IL6, PAI1, and SDF1, is strongly up-regulated in iPSC-MSCs (29), conditions that were previously reported to produce TGFBR2 and IL6 produced by cancer cells or tumor stromal cells (29) and passaged at 80 receptors (SDF13).

In summary, compared with BM-MSCs, iPSC-MSCs developed with our modified protocol have the same tumor tropism but much less protumor potential. They can also be readily genetically engineered and the protocol can be scaled up to produce large numbers of the cells. Hence, iPSC-MSCs prepared with the modified protocol provide a promising alternative to BM-MSCs for therapy of cancer patients or survivors and for other applications including bioengineering.

Materials and Methods

Detailed information is provided in SI Materials and Methods.

CY2 iPSCs were maintained and expanded in Matrigel-coated plates in the feeder-free mTeSR1 medium (Stemcell Technologies). For MSC derivation, iPSCs were first cultured in the mTeSR1 medium supplemented with 10 µM TGFβ inhibitor SB-431542 (44) (Sigma-Aldrich) in Matrigel-coated plates at 37 °C and 7.5% CO₂ (29) and passaged at 80–90% confluence by 2 mg/mL of Dispase. When most cells at the edge of a cell cluster became spindle-shaped in about 25 d, they were trypsinized into single cells and cultured in standard tissue-culture plastic dishes with modified human ESC-MSC medium (30) in the presence of SB-431542. The medium was changed daily, and the cells were passaged at 80–90% confluence at the ratio of 1:3 about every 3 d and analyzed for expression of MSC surface markers by flow cytometry weekly. When the majority of cells were positive for MSC surface markers, they were

![Fig. 5. TGFβ-SDF1 pathway in iPSC-MSCs. (A) qRT-PCR analysis of TGFβ receptors (TGFBR1 and TGFBR2) and TGFβ target genes (ID3, IL6, PAI1, and SDF1) in MSCs cultured with mEMEM, TGFβ, or MDA-MB-231-conditioned medium (TCM) for 3 d. (B) Western blot analysis of levels of phospho-Smad2 and phospho-Smad3 in MSCs cultured under above conditions, normalized to GAPDH (n = 3).](https://example.com/f5.png)

![Fig. 6. The expression of HASs and TSG6 and the HA production in MSCs and the induction of LOX in cancer cells. (A) qRT-PCR analysis of HAS1-3 and TSG6 in MSCs cultured alone or with LoVo cells for 3 d. (B) Enzyme immunoassay (EIA) analysis of HA in 6-d medium of BM-MSCs or iPSC-MSCs at passages 5 and 15. (C) qRT-PCR analysis of LOX in cancer cells cultured alone or with MSCs for 3 d.](https://example.com/f6.png)

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assays, breast cancer cells were transduced with CMV-copGFP and cultured
(Allied Biotech, MT3010). A CFU assay and migration assays of MSCs were performed as reported (37). Invasion assays were performed with 8-μm porous membranes coated with collagen IV. ALDH activity was examined with the ALDEFLUOR kit (Stemcell Technologies). For mammosphere assays, breast cancer cells were transduced with CMV-copGFP and cultured alone or with BM-MSCs or iPSC-MSCs for 3 d, isolated by FACS, and then incubated for 7 d at 500 cells/well in ultra-low-attachment 12-well plates. All animal work was approved by the joint Scott & White and Texas A&M Health Science Center Institutional Animal Care and Use Committee. When s.c. xenograft models of LoVo or MDA-MB-231 cancers were established, BM-MSCs or iPSC-MSCs transduced with CMV-copGFP lentiviruses were injected via tail vein, and tumors were harvested 16 h later for section and qPCR assay of CMV promoter (50). For tumor initiating and growth assay, HCC1806 cancer cells were co-injected with BM-MSCs or iPSC-MSCs into the fourth mammary fat pad of NOD SCID mice, and tumors were harvested 6 wk after inoculation.

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