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Advances in Brief

Bone Marrow-derived Mesenchymal Stem Cells as Vehicles for Interferon-β Delivery into Tumors[1]

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Abstract

Molecules that physiologically control cell proliferation are often produced locally in tissues and are rapidly destroyed when they enter circulation. This allows local effects while avoiding interference with other systems. Unfortunately, it also limits the therapeutic use of these molecules via systemic delivery. We here demonstrate that, for the purpose of anticancer therapy, bone marrow-derived mesenchymal stem cells (MSCs) can produce biological agents locally at tumor sites. We show that the tumor microenvironment preferentially promotes the engraftment of MSCs as compared with other tissues. MSCs with forced expression of IFN-β inhibited the growth of malignant cells *in vivo*. Importantly, this effect required the integration of MSCs into the tumors and could not be achieved by systemically delivered IFN-β or by IFN-β produced by MSCs at a site distant from the tumors. Our results indicate that MSCs may serve as a platform for delivery of biological agents in tumors.

Introduction

Cells in most if not all tissues are hierarchically organized with regard to their proliferative and differentiation potential (1). This hierarchy is fully operational in tissues with high spontaneous turnover such as blood, skin, and gut. In these tissues, short-lived, terminally differentiated cells are continuously replaced from undifferentiated precursors that are maintained from a compartment of self-renewing stem cells. In contrast, the turnover of connective tissue is low, and its hierarchical organization (2) only becomes apparent when the demand for new functional cells is increased, such as during wound healing or regeneration after injury. Bone marrow-derived MSCs[3] are precursors with high proliferative capacity (3) and can differentiate into adipocytes, chondrocytes, osteoblasts (4), and possibly other cell types (5). Recent data from animal experiments (6) and a clinical trial (7) indicate that the conditions characterized by increased cell turnover and tissue remodeling, such as multiple bone fractures in metabolic bone disease or the rapidly growing embryo during prenatal development, provide effective signals necessary for survival and proliferation of systemically delivered MSCs. In a related sense, tumor growth requires formation of supportive mesenchymal stroma (8). The process of tumor stroma formation is similar to wound healing (9) and results in tissue remodeling with high proliferation of mesenchymal cells (10, 11). Therefore, we hypothesized that exogenously administered MSCs would preferentially engraft at the tumor sites and contribute to the population of stromal fibroblasts. This may allow development of a therapeutic strategy based on the local production of biological agents in tumors by gene-manipulated MSCs. For that purpose, we transduced MSCs with IFN-β and investigated whether they inhibit growth of malignant cells *in vitro* as well as *in vivo*.

Materials and Methods

Cell Isolation and Culture. Human MSCs were isolated from the bone marrow of normal individuals undergoing bone marrow harvest for allogeneic bone marrow transplantation after informed consent according to institutional guidelines under the approved protocol. Mononuclear cells were separated by centrifugation over a Ficoll-Hypaque gradient (Sigma Chemical Co., St. Louis, MO) and suspended in α-MEM medium containing 20% fetal bovine serum (Life Technologies, Inc., Rockville, MD), L-glutamine and penicillin-streptomycin mixture (Flow Laboratories, Rockville, MD), followed by plating at an initial seeding density of 1×10^6 cells/cm^2. After 3 days, the nonadherent cells were removed by washing with PBS, and monolayers of adherent cells were cultured until they reached confluence. Cells were then trypsinized (0.25% trypsin with 0.1% EDTA), subcultured at densities of 5000–6000 cells/cm^2, and used for experiments during passages three to four.

The A375SM cell line was established in culture from lung metastases produced by A375P cells growing s.c. in nude mice (12). Cells were maintained in α-MEM with 10% FCS, sodium pyruvate, nonessential amino acids, L-glutamine, vitamin solution (Life Technologies, Inc., Grand Island, NY), and a penicillin-streptomycin mixture.

Adenoviral Vectors and MSC Transduction. The adenoviruses were created using the bacterial plasmid recombination system AdEasy (Qbiogene). Briefly, the gene for β-gal was cloned into the *Not*I/*Hin*dIII-digested adenoviral cytomegalovirus shuttle. The gene for human IFN-β was purchased from InvivoGen (San Diego, CA), digested with *Cla*I, and filled in to achieve a blunt end. This blunt-ended plasmid was further digested with *Bgl*II to release the 570-bp fragment containing human IFN-β, and this piece subcloned into the *Bgl*II/*Eco*RV sites of pShuttle cytomegalovirus. These two clones were sequenced to determine the correct reading frame and any possible mutations. The two plasmids were linearized with *Pme*I, dephosphorylated using calf-alkaline phosphatase, extracted with two rounds of phenol chloroform, and mixed with *Pac*I-digested pAdEASY-1. These two linearized plasmids were electroporated into bacteria and plated on Kan+ agar, and kanamycin-resistant clones were picked and analyzed for AdEasy sequences. We identified four clones of each gene (β-gal and IFN-β), and these plasmids were expanded in a 3-ml miniprep format and transfected into 293 cells using Fugene6. Eighteen to 20 days later, plaques were eluted, and recombinant virus was rescued from the cultures. We performed two rounds of amplification, and viruses expressing IFN-β as identified by ELISA (Fujirebio, Inc., Tokyo, Japan) or expressing β-gal (as detected by histochemical staining) were chosen. These viruses were

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[3] The abbreviations used are: MSC, mesenchymal stem cell; β-gal, β-galactosidase; Sp-DiI, dioctadecyl-di(sulfophenyl)-tetramethyl-indocarbocyanine; BrdUrd, bromodeoxyuridine.

used in subsequent experiments. MSCs were incubated with adenoviruses at a multiplicity of infection of 3000 for 2 h. MSCs produced 3–4 $\times$ 10^4 IU of IFN-β per 10^6 MSCs during the first 24 h after infection. β-gal expression in MSCs was determined by histochemical stain, and >90% of MSCs were positive.

Coculture of A375SM Melanoma Cells with MSC *in Vitro*. A375SM melanoma cells (5×10^4 per well) were cultured either alone or mixed with various numbers of MSCs and IFN-β-MSCs, respectively (5×10^3, 10^4, and 2.5×10^5 per well) in six-well plates for 72 h. Cells were then trypsinized, counted, and fixed with 70% ethanol. The relative numbers of MSCs (diploid cells) and A375 cells (aneuploid cells) were determined using ModFit software (Verity Software House, Inc., Topsham, ME) after fixation with 70% ethanol, labeling the cells with propidium iodide (Sigma), and analyzing DNA content using the FACScan flow cytometer (Becton-Dickinson, San Jose, CA).

MSC Labeling with the Fluorescent Dye SP-DiI. The fluorescent dye SP-DiI (Molecular Probes, Eugene, OR) was dissolved in dimethylformamide (Sigma) to the concentration of 2.5 mg/ml. SP-DiI dye was then added directly to culture medium to a final concentration of 10 μg/ml. MSCs (4×10^6 cells) were incubated with 25 ml of medium with SP-DiI in T175 flask for 48 h. Then, cells were washed with PBS, incubated with dye-free medium for 4 h and used for experiments.

Animals and Cell Administration. Male athymic nude mice (NCr-nu) were purchased from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research and Development Center (Frederick, MD). The mice were used in accordance with institutional guidelines under the approved protocols. Cells were administered as suspension in 100 μl of PBS either s.c. into the flank or i.v. into the lateral tail vein.

Tumor Measurements and Determination of Animal Survival. Tumors were measured by caliper, and tumor area was calculated as the geometric mean of two perpendicular diameters. Survival was measured from the day of A375SM cell injection to death, or when the mouse had to be sacrificed secondary to tumor diameter >15 mm, tumor ulceration, or bleeding. The difference in survival was determined by log rank test.

Tissue Processing and Imaging Studies. Tumors and other organs were embedded in OTC compound (Miles, Inc., Elkhart, IN), snap frozen in liquid nitrogen, and stored at −70°C. Tissue was sectioned (6–8 μm) and processed for H&E staining, fluorescence microscopy, or immunohistochemistry. Each slide was first imaged with fluorescence microscopy and then fixed and stained for immunohistochemistry. Imaging was performed with a Ziess Axioplan2 microscope (Carl Zeiss, Inc., Thornwood, NY) equipped with a CCD camera (Hamamatsu Corp., Bridgewater, NJ) and Adobe Photoshop software (Adobe Systems, Inc., San Jose, CA). The percentage of MSC-derived cells in lung tumors was determined as number of AS02-positive cells to all nuclei counterstained with Gill's hematoxylin per field (×100). At least five fields from tumor areas that were visually judged to express the highest number of positive cells were chosen, and 5 positive tumors at each time point were evaluated. Results were expressed as mean ± SE.

Imunohistochemistry with AS02 Antibody. Slides were fixed in cold acetone, and endogenous peroxidase was blocked by 3% hydrogen peroxide in methanol. Nonspecific binding was blocked by incubation with F(ab_2) IgG fragment of goat antimouse antibody (Jackson, West Grove, PA; dilution 1:10), 5% horse serum, and 1% goat serum in PBS for 24 h at 4°C. Primary mouse antihuman AS 02 antibody (Dianova, Inc., Hamburg, Germany; dilution 1:20) was used overnight at 4°C, followed by peroxidase-conjugated rat antimouse IgG1 antibody (PharMingen, San Diego, CA; dilution 1:600) for 1 h at room temperature. Positive reaction was visualized with stable 3,3′-diaminobenzidine (Research Genetics, Huntsville, AL).

Immunofluorescence Staining for BrdUrd. Two hundred μl of 10 mM BrdUrd (Sigma) dissolved in PBS was administered i.v. 4 and 2 h before animals were sacrificed. Slides were fixed with 4% paraformaldehyde, treated with 0.1% Triton X-100 in PBS, incubated with 2 N HCl for 30 min at 37°C, and washed with 0.1 M Tris. Then, slides were incubated with primary mouse anti-BrdUrd antibody (Becton Dickinson, Mountain View, CA; dilution 1:100) overnight at 4°C, followed by secondary goat antimouse Alexa 488 antibody (Molecular Probes, Eugene, OR; dilution 1:400) for 1 h at room temperature, and mounted in mounting medium (Vector).

Results

We have investigated the interaction of human bone marrow-derived MSCs with A375SM melanoma cells of human origin in a mouse xenograft model. MSCs were labeled with the fluorescent dye SP-DiI, premixed with A375SM melanoma cells, and injected s.c. into nude mice. Tumors ($n = 10$) were then examined by fluorescence microscopy and immunohistochemistry with an antibody specific for human fibroblasts (13) that does not cross-react with mouse tissue or human melanoma cells (Fig. 1). These studies revealed that a significant portion of MSC-derived fibroblasts was incorporated into the tumor architecture and formed a fibrous capsule at the tumor periphery (Fig. 1, *A–C*). Many of the MSC-derived fibroblasts in the tumor capsule lost SP-DiI fluorescence (Fig. 1*B*). Because SP-DiI is tightly bound to the cellular membrane and is not transferred to neighboring cells *in vivo* (14), the fluorescence intensity of labeled MSCs declines only during cell division when the membrane-bound dye of the parental cell is evenly distributed between both daughter cells. Repeated cell divisions lead to a further decrease of the fluorescence signal until it is indistinguishable from the background of surrounding unlabeled cells. Therefore, we concluded that the observed loss in fluorescence intensity of MSC-derived cells in the tumor capsule may be related to their proliferation and cell division.

Direct evidence of MSC proliferation in tumors was obtained from *in vivo* BrdUrd labeling. Mice with tumors derived from mixtures of melanoma cells and SP-DiI-labeled MSCs ($n = 4$) were i.v. injected with BrdUrd. Proliferating cells were identified by BrdUrd immunofluorescence (Fig. 1, *H* and *I*). This method clearly showed SP-DiI-labeled MSCs with BrdUrd-positive nuclei in tumors (Fig. 1*H*). In contrast, BrdUrd was not incorporated into MSCs injected s.c. alone without A375SM melanoma cells (Fig. 1*I*). Our results suggest that bone marrow MSCs contribute to tumor stroma formation when coinjected with the malignant cells. This process involves not only passive incorporation of MSCs into the tumor architecture but also their proliferation.

Next, we examined whether MSCs contribute to tumor stroma formation after i.v. administration. Mice with established A375SM melanomas growing in the lungs ($n = 9$) were injected with MSCs through the tail vein and then sacrificed after 1, 8, and 60 days. The distribution of MSC-derived cells in melanoma nodules and lung parenchyma was then examined by immunohistochemistry (Fig. 2). MSCs were randomly distributed in lung parenchyma and tumor nodules 1 day after their i.v. administration (Fig. 2, *A–C*). However, after 8 days, MSCs were found mainly in tumors and had cleared from normal lungs (Fig. 2, *D* and *E*). Similarly, MSCs were detected in tumors (Fig. 2, *F* and *G*) but not in lung parenchyma 60 days after injection (Fig. 2*H*). The preferential distribution of MSCs in tumors but not lungs at the latter time points indicates that tumor microenvironment but not normal lung parenchyma supports their survival and incorporation into stroma. Furthermore, the percentage of MSCs

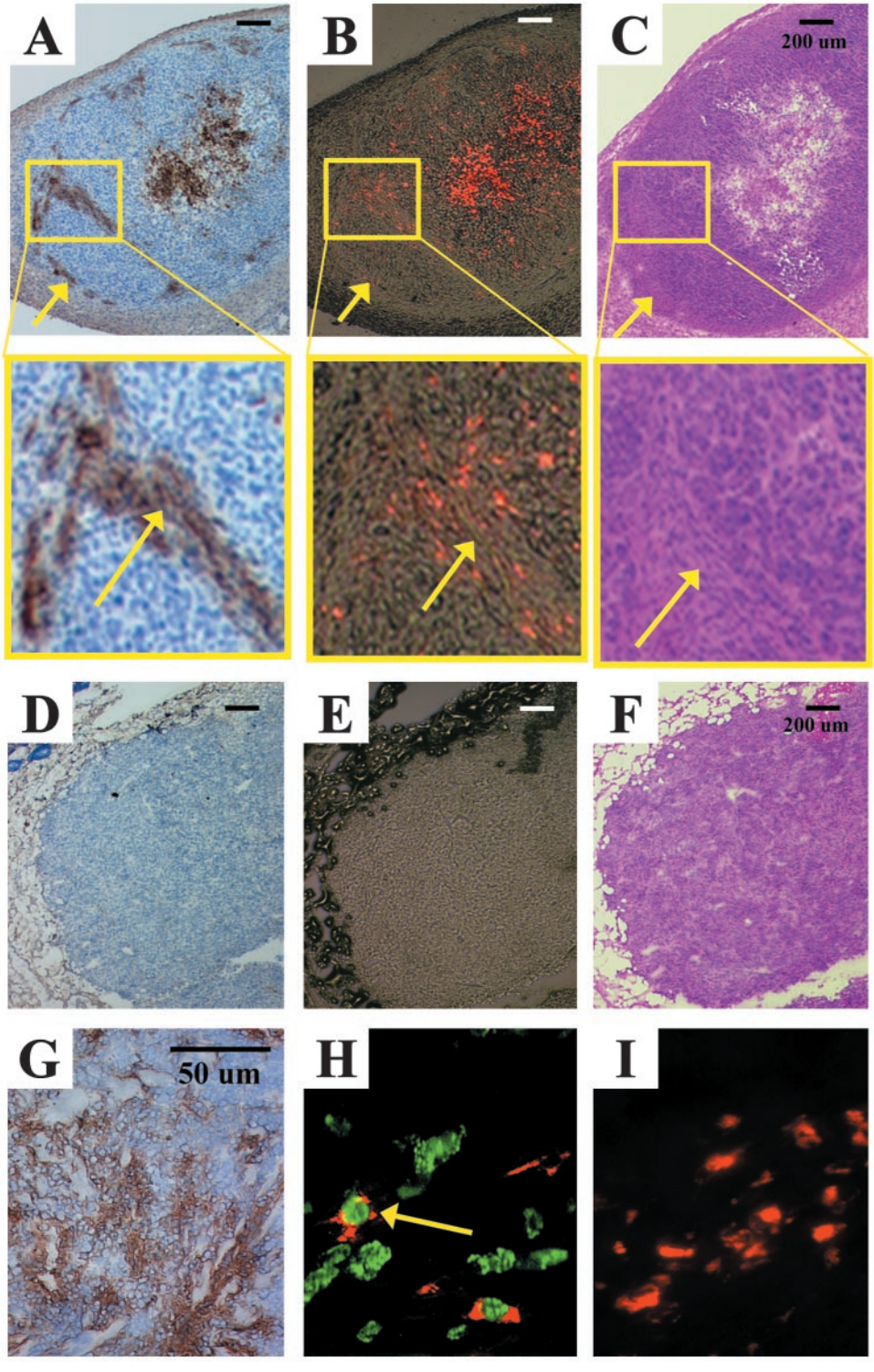


Fig. 1. Malignant cells induce the proliferation of MSCs. Fluorescent dye-labeled human MSCs were coinjected with A375SM melanoma cells s.c. into nude mice (*A–C*). The distribution of MSC-derived fibroblasts in tumors is shown by immunohistochemistry (*A*) and fluorescence microscopy (*B*). Tumor morphology is visualized by H&E (*C*). Numerous MSC-derived fibroblasts were detected by immunohistochemistry at the tumor periphery (*arrows* in *A*) and correspond to the fibrous capsule (*arrows* in *C*). Some MSCs in the capsule remain highly fluorescent, whereas others display significantly lower fluorescence intensity (*arrows* in *B*). Tumors formed by A375SM cells injected into mice without human MSCs (containing stromal fibroblasts of mouse origin) were used as negative controls (*D–F*), and primary human melanomas from patients containing stromal fibroblasts of human origin served as positive controls (*G*). MSC proliferation in tumors (*arrows* in *H*) is confirmed by BrdUrd incorporation (*green*) into the fluorescence-labeled MSCs (*red*). In contrast, BrdUrd incorporation was not observed when MSCs were injected alone without the malignant cells (*I*).

in tumors was approximately stable during the experiment (day 1, 3% ± 2%; day 8, 11% ± 2%; and day 60, 5% ± 1%). Because the tumors size increased between days 1 and 60 (Fig. 2, *A*, *D*, and *F*), the absolute number of MSC-derived cells in individual tumor nodules should also have increased during this time, presumably by proliferation.

We further investigated whether i.v. injected MSCs can integrate into s.c. tumors. Mice with A375SM melanomas received five doses (10^6 cells/dose) of unlabeled MSCs over a 20-day period. Mice ($n = 10$) were sacrificed 15–20 days later, and tumors, livers, spleens, and lungs were evaluated by immunohistochemistry. MSC-derived fibroblasts were consistently identified in 50% of tumors (Fig. 2, *I*, *J*, and *K*) but were not found in other organs, except for the rare positive cells seen in the spleens of some animals.

We then examined the therapeutic potential of MSCs as cellular vehicles for production of anticancer agents after their transduction with an adenoviral vector carrying the human *β-IFN* gene. We determined the effect of IFN-β producing MSCs (IFN-β-MSC) on

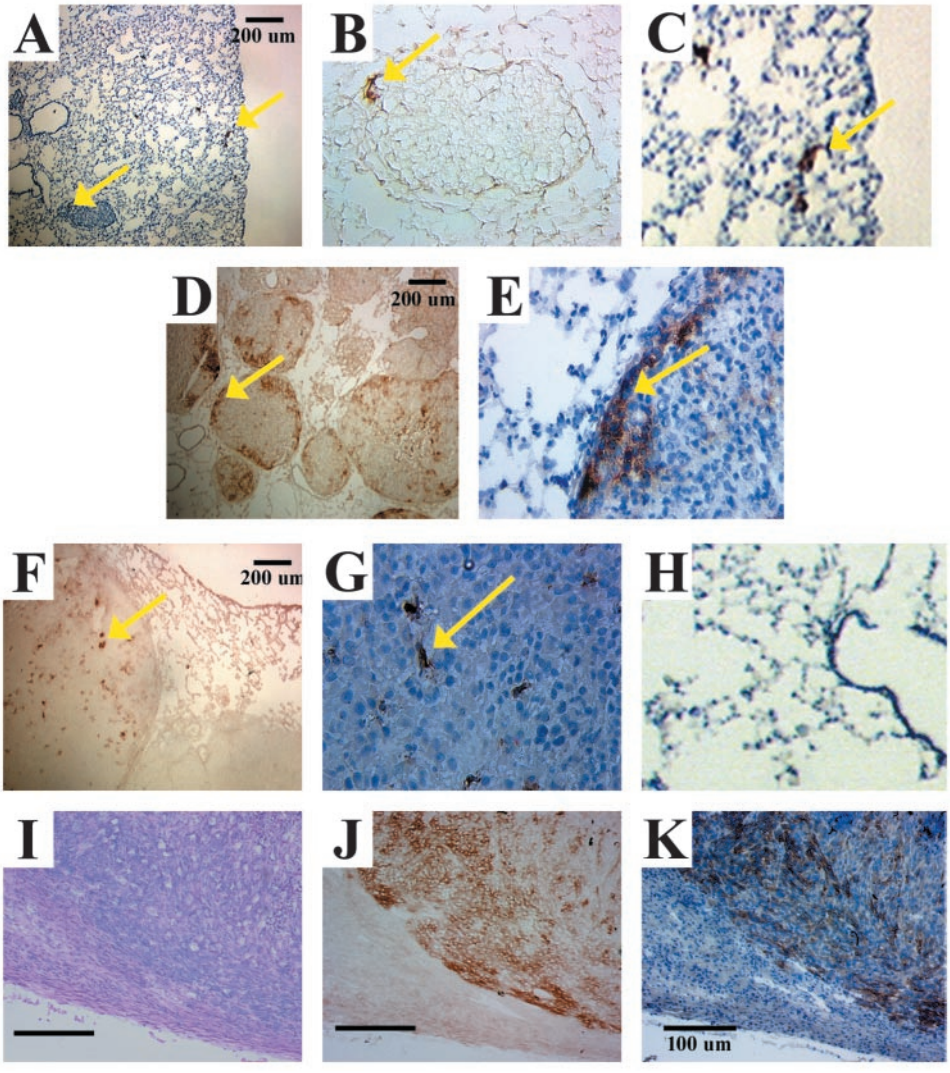


Fig. 2. i.v. administered MSCs integrate into tumors. MSCs were injected i.v. into mice with A375SM melanomas growing in the lungs. Tissues were examined after 1 day (*A–C*), 8 days (*D* and *E*), and 60 days (*F–H*). After 1 day, MSCs were randomly distributed in lungs and tumors (*arrows* in *A–C*). After 8 days, MSCs were seen preferentially at the tumor periphery (*arrows* in *D* and *E*). After 60 days, numerous MSCs were diffusely present in the tumors (*arrows* in *F* and *G*) but not in lungs (*H*). Note also the increasing tumor size at the later time points (*A*, *D*, and *F*). Slides *A*, *C*, *E*, *G*, and *H* were counterstained with Gill's hematoxylin; *B*, *D*, and *F* were not counterstained. s.c. growing A375SM melanomas after the i.v. injections of MSCs stained with H&E (*I*), immunohistochemistry for MSC-derived fibroblasts (*J*), immunohistochemistry for MSC-derived fibroblasts with counterstained nuclei (*K*) are shown. MSC-derived fibroblasts closely followed the distribution of malignant cells and were not detected outside the tumors.

A375SM melanoma cells in a coculture system under *in vitro* conditions (Fig. 3). These experiments indicated that IFN-β-MSCs directly inhibited the growth of malignant cells and did not require the host immune system for this effect.

A375SM melanoma cells (10^6 cells) were coinjected s.c. into nude mice together with 5×10^5, 10^5, or 10^4 IFN-β-MSCs at the same site. These numbers represented 50, 10, and 1% of malignant cells and corresponded to the frequency of MSCs found in tumors in biodistribution studies (3–11% of all cells in tumors). IFN-β-MSCs suppressed tumor growth and prolonged the life of the animals in all of these groups (Fig. 4, *A* and *C*). Of note, even 1% of IFN-β-MSCs (10^4 cells) were able to exert control of tumor growth (Fig. 4*A*) and result in significant prolongation of survival (Fig. 4*C*). In contrast, IFN-β produced by a 50 times higher number of IFN-β-MSC (5×10^5 cells) injected s.c. into the flank contralateral to the site of the tumor or the s.c. administration of human IFN-β [5×10^4 IU of Avonex (Biogen, Inc.) administered every other day] did not have any effect on tumor growth (Fig. 4*B*) or survival (Fig. 4*C*). These data suggest that local IFN production in the tumor microenvironment is essential for control of malignant cells and cannot be substituted for by corresponding systemic levels of IFN-β in serum delivered from a distant site.

We next extended this approach to a clinically relevant situation and examined the efficacy of i.v. administered IFN-β-MSCs in a pre-established metastatic melanoma model (Fig. 4*D*). Tumor nodules were allowed to develop in the lungs of mice injected i.v. with A375SM melanoma cells, after which the animals received the same number of IFN-β-MSCs via one of two different routes. One group received IFN-β-MSCs as an i.v. injection through the tail vein and another group as s.c. injection into the flank. On the basis of our distribution data, we anticipated that i.v. injected MSCs would freely travel via the blood stream, become incorporated into the tumor stroma, and produce IFN-β locally in the tumor microenvironment. Conversely, s.c. injected IFN-β-MSCs would not migrate from the site of injection and produce systemic levels of IFN-β. Local production of IFN-β in tumors resulting from i.v. injected IFN-β-MSCs did significantly prolong animal survival ($P = 0.023$; Fig. 4*D*). In contrast, the systemic levels of IFN-β supplied by the same number of s.c. injected IFN-β-MSCs had no effect ($P = 0.21$; Fig. 4*D*). As expected, the tumor inhibition in our experiments was not permanent and corresponded to the relatively short-lived IFN-β expression characteristic of conventional adenoviral vectors. However, this hurdle could be overcome by a stable transfection system with regulated protein expression.

Discussion

We demonstrated that exogenously administered MSCs preferentially survive and proliferate in the presence of malignant cells and

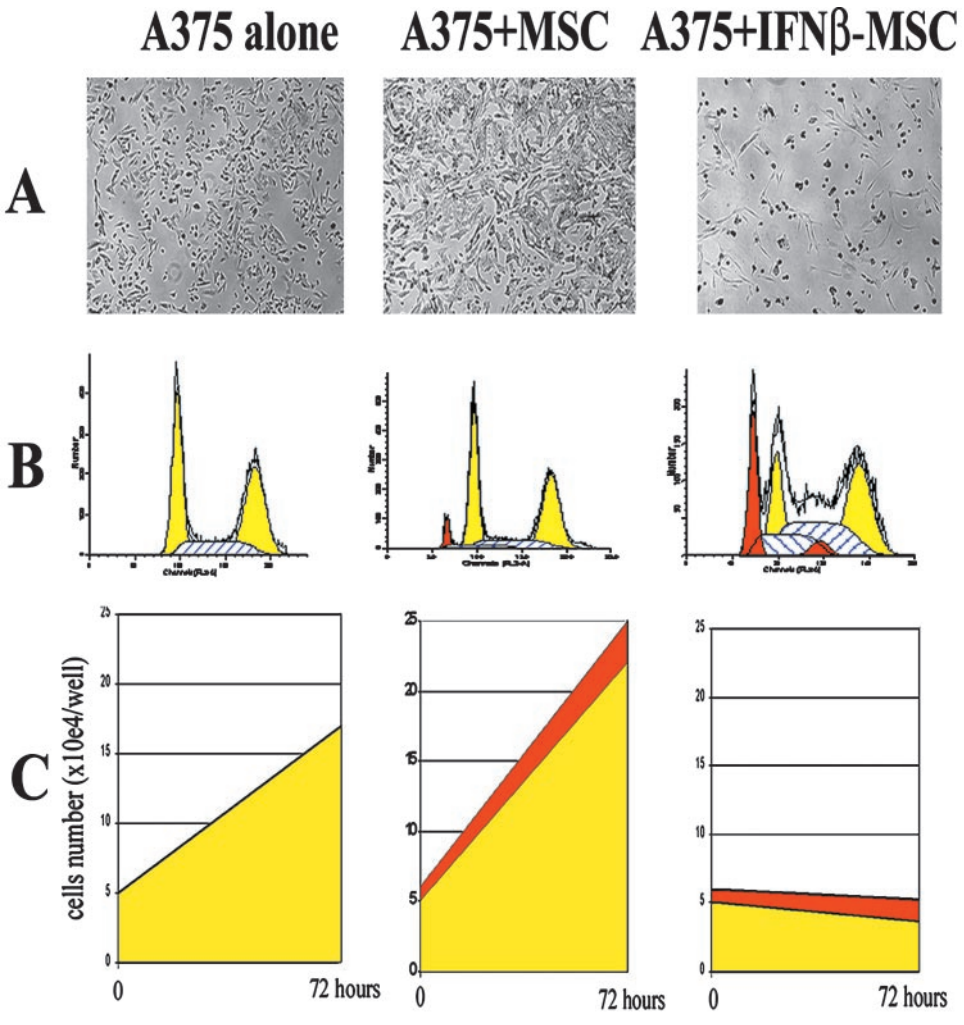


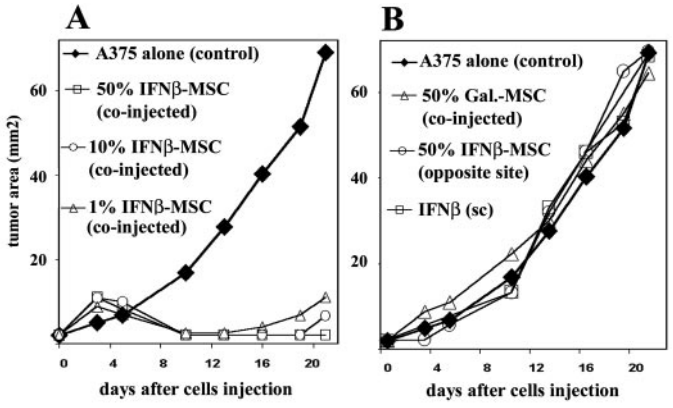
Fig. 3. MSCs producing IFN-β (*IFN-β-MSC*) inhibit the growth of A375SM melanoma cells *in vitro*. A375SM melanoma cells were either cultured alone or cocultured with unmanipulated MSCs and IFN-β-MSC for 72 h (*A*). Numbers of diploid MSCs (*red*) and aneuploid A375SM melanoma cells (*yellow*) were determined by flow cytometry (*B*) and cell counting. IFN-β-MSCs directly inhibited the growth of A375SM melanoma cells as compared with A375SM cells alone or A375SM cells cocultured with untransduced MSCs (*C*).

become incorporated into the tumor architecture as stromal fibroblasts. This process could be related to high local concentrations of paracrine growth factors such as fibroblast growth factor, platelet-derived growth factor, epidermal growth factor, transforming growth factor-β, or other mediators within the tumor microenvironment (8). It has been demonstrated, at least *in vitro*, that MSC proliferation depends on adequate concentrations of these molecules (15).

Our findings do not contradict the traditional view that tumor stromal fibroblasts are recruited from locally resident precursors (16). Nevertheless, we demonstrated that MSCs compete with local mesenchymal precursors in their ability to proliferate once placed into the tumor environment. Interestingly, the possibility that reactive fibroblasts in healing wounds originate from local as well as remote sources has been demonstrated recently (17). Moreover, it has been shown that human bone marrow MSCs preferentially engraft at the site of wounds after systemic administration into fetal sheep (6, 18). It remains unclear whether bone marrow cells developing into MSCs under *in vitro* conditions are identical to circulating mesenchymal precursors in the peripheral blood (19). We assume that cultured bone marrow adherent cells, whether appropriately or inappropriately called MSCs, contained cells with extraordinary high proliferative capacity that can potentially contribute to the maintenance of connective tissue in organs remote from the bone marrow (20). However, disturbance in tissue steady-state that leads to the increased cells turnover is probably essential for successful engraftment of MSCs in tissues. This process can be triggered by tissue damage or, in our case, by tumor growth. We chose MSCs, as opposed to other sources of progenitor cells, because of their accessibility for genetic manipulation *in vitro*.

We explored the therapeutic potential of MSCs as a delivery system into the tumor microenvironment by their transduction with human *IFN-β* gene. IFN-β has a wide range of biological activities and can induce tumor regression through indirect immunomodulatory (21) and antiangiogenic properties or through direct antiproliferative effects on malignant cells (22). We confirmed that IFN-β-MSCs directly control the proliferation of melanoma cells *in vitro* (Fig. 3) and do not require the immune system for this effect. Moreover, human IFN-β produced by IFN-β-MSCs is species specific and does not directly influence endothelial cells or residual immune cells of mouse origin (23). Therefore, we assume that the tumor suppression seen in our *in vivo* model was related to the direct antiproliferative action of human IFN-β-MSCs on human tumor cells.

Clinical studies (24) have shown that the serum concentrations of IFN-β after systemic administration of the maximally tolerated dose is far below that required to achieve the antiproliferative effects observed *in vitro*. This suggests that a direct antiproliferative effect of IFN-β on malignant cells rarely if ever occurs in patients and may explain the disappointing efficacy of this biological agent in clinical trials (25). Our results indicate that the local production of IFN-β by MSCs in the tumor microenvironment can overcome this limitation and simulate the physiological role of IFN-β as a short-range paracrine regulator of cell proliferation and differentiation (26). Under



C

	Survival in days median (range)	P value*
A 375 alone (control)	26 (21-31)	
50% Gal.-MSC (co-injected)	24 (24-27)	0.388
50% IFNβ-MSC (opposite site)	24 (24-27)	0.420
IFNβ (sc)	26 (24-27)	0.480
1% IFNβ-MSC (co-injected)	41 (41-67)	0.014
10% IFNβ-MSC (co-injected)	56 (41-67)	0.015
50% IFNβ-MSC (co-injected)	59 (59-110+)#	0.010

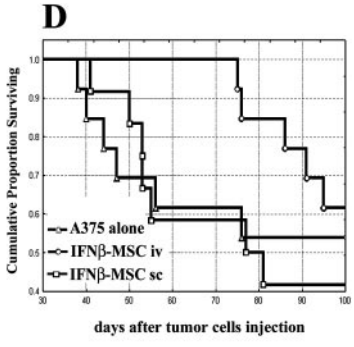


Fig. 4. Local production of IFN-β by IFN-β-MSCs in tumors but not systemic IFN-β is effective in inhibiting tumor growth *in vivo*. IFN-β-MSCs were either coinjected s.c. together with 10^6 A375SM melanoma cells at the same site, or the cells were injected s.c. into two separate sites (opposite flank of the animals). Tumor growth was inhibited (*A*), and the survival of animals was prolonged (*C*) only after the coinjection of A375SM melanoma cells with IFN-β-MSCs at the same site. Coinjected IFN-β-MSCs were effective at doses representing 1, 10, or 50% of the initial malignant cells number. However, systemic levels of IFN-β produced by the highest number of IFN-β-MSCs (50%) injected s.c. into a remote site (the side contralateral to the tumor) or the s.c. administration of a corresponding dose of IFN-β (5×10^4 IU every other day) had no effect. Similarly, the coinjection of melanoma cells with 50% MSCs transduced with control adenovirus carrying β-galactosidase gene (*Gal.*) was not effective. Difference in survival was compared by log rank test (*). Two animals (#) were alive and free of tumors 150 days after cell injection. The tumor size is a mean of 5 animals per group. *D*, survival of mice with A375SM metastatic melanomas in their lungs after either i.v. or s.c. injection of IFN-β-MSCs. Mice were i.v. injected with 10^6 A375SM melanoma cells and after 10 days started receiving four weekly doses of 10^6 IFN-β-MSCs either i.v. or s.c. Animals were observed until death resulting from melanoma metastasis in the lungs. IFN-β-MSCs injected i.v. produced IFN-β locally in lung tumors, and this was associated with significantly prolonged survival ($P = 0.021$). Conversely, the systemic levels of IFN-β in serum supplied from the same numbers of IFN-β-MSCs injected s.c. were not effective ($P = 0.4$). There was a significant difference in the survival of animals with i.v. injected IFN-β-MSCs as opposed to s.c. injected IFN-β-MSCs ($P = 0.023$). There were no additional deaths after day 100 of the experiments, and all remaining animals sacrificed at 130 days were tumor free.

physiological conditions, IFN-β is produced by cells to influence neighbors spatially located in the same area and, at the same time, avoid interference with regulatory mechanisms that control cells in other areas of the body. Therefore, perhaps, the systemic administration of IFN-β cannot attain this physiological function. These arguments served as the rationale for the use of IFN-β in our studies. However, the same approach could be explored in the delivery of other agents with antitumor activities.

Overall, we have demonstrated that MSCs could serve as a powerful cellular delivery system for the local production of anticancer therapeutics in tumors. We believe that this approach will be useful not only as a means of improving the pharmacokinetics of various biological agents but also as a more general tool for modifying the microenvironment in neoplasms.

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