Abstract. Background/Aim: The effects of adipose tissue-derived mesenchymal stem cells (AT-MSCs) on the growth of human malignancies, including melanoma, are controversial and the underlying mechanisms are not yet-well understood. The aim of the present study was to investigate the in vitro and in vivo anti-tumor effects of human AT-MSCs on human melanoma. Materials and Methods: The inhibitory effect of AT-MSC-conditioned medium (AT-MSC-CM) on the growth of A375SM and A375P (human melanoma) cells was evaluated using a cell viability assay. Cell-cycle arrest and apoptosis in melanoma cells were investigated by flow cytometry and western blot analysis. To evaluate the in vivo anti-tumor effect of AT-MSCs, CM-DiI-labeled AT-MSCs were circumtumorally injected in tumor-bearing athymic mice and tumor size was measured. Results: AT-MSC-CM inhibited melanoma growth by altering cell-cycle distribution and inducing apoptosis in vitro. AT-MSCs suppressed tumor growth in tumor-bearing athymic mice and tumor size was measured. AT-MSC-CM inhibited melanoma growth by altering cell-cycle distribution and inducing apoptosis in vitro. AT-MSCs suppressed tumor growth in tumor-bearing athymic mice and tumor size was measured. Conclusions: AT-MSCs inhibit the growth of melanoma suggesting promise as a novel therapeutic agent for melanoma.

Although melanoma accounts for less than 10% of all skin cancers, malignant melanoma is an aggressive disease that accounts for 75% of skin cancer-related deaths (1). The incidence rates of melanoma in the USA have been continuously increasing in the last few decades, with the incidence estimated to be 76,690 new melanoma cases and 9,480 related deaths in 2013 (2). Current therapy includes surgery, radiotherapy and chemotherapy. Once the disease becomes metastatic, these treatments are rarely curative and have little benefit, and they do not result in an improvement in overall survival (3). For these reasons, several advanced therapeutic strategies have been investigated (3-5) as there is definitely an urgent need for novel treatment options with better efficacy.

Mesenchymal stem cells (MSCs) are a fibroblast-like subset of stromal stem cells that can differentiate into bone, cartilage and fat cells. MSCs have been isolated from many adult tissues including: bone marrow, brain, liver, pancreas, skin, adipose tissue, umbilical cord, Wharton’s jelly and placenta (6-9). Adipose tissue-derived mesenchymal stem cells (AT-MSCs) were first isolated by Zuk et al. (10). These adult stem cells share similar characteristics to bone marrow-derived MSCs (BM-MSCs) with regard to morphology and their ability to undergo differentiation into multiple cell types (10). Adipose tissue can be obtained by less invasive procedures and in larger quantities compared to bone marrow. The yield of MSCs from adipose tissue is about 40-fold higher than the yield from bone marrow (11). These features make adipose tissue an attractive candidate for clinical and therapeutic use.

MSCs have the ability for self-renewal and differentiation into multiple cell types and they, therefore, hold great promise for tissue repair and regenerative medicine. In recent years, MSCs have been receiving increased attention because they were shown to be capable of migrating towards tumor sites. This property has led to the use of MSCs as vehicles to deliver therapeutic agents, such as cytokines, apoptosis inducers, prodrugs and interferons to tumor sites for growth inhibition (12-16). Furthermore, MSCs genetically engineered to produce antitumor molecules have shown strong therapeutic effects in experimental melanoma models (15-17). However, very few studies have investigated the antitumor properties of MSCs themselves and their impact on tumor progression is still under debate. Some studies have suggested that MSCs inhibit tumor growth (18, 19), while others believe that MSCs promote tumor progression and metastasis (20, 21). For example, Sun et al. reported that BM-MSCs promoted proliferation of tumor.
cells and improved the microenvironment in B16 mouse melanoma cells (22). Thus, the antitumor effect of MSCs on the targeted tumor should be further investigated prior to their use as delivery vehicles for tumor-targeted gene therapy.

In the present study, we investigated the in vitro and in vivo anti-tumor potential of AT-MSCs in melanomas. We found that AT-MSC-conditioned medium (AT-MSC-CM) suppresses melanoma proliferation and can significantly induce cell-cycle arrest and apoptosis in the A375SM and A375P melanoma cell lines. We also observed that treatment with AT-MSCs reduced the tumor volume in melanoma-bearing athymic nude mice. Our findings suggest that AT-MSCs have favorable anticancer characteristics and should be further explored in future studies on melanoma therapy.

**Materials and Methods**

**AT-MSC isolation and culture.** Human adipose tissue samples were obtained by liposuction from abdominal subcutaneous fat after informed consent of the donors was obtained. AT-MSCs were prepared in accordance with the Good Manufacturing Practice guidelines (K-STEMCELL CO. Ltd., Seoul, Korea) as described previously (23). Briefly, subcutaneous adipose tissues were digested with collagenase I (1 mg/ml) under gentle agitation for 60 min at 37°C. The digested tissues were filtered through a 100-μm nylon sieve to remove cellular debris and were collected by centrifugation at 470 x g for 5 min. The cell pellet was re-suspended in RCME (K-STEMCELL media for MSC attachment, K-STEMCELL) containing 10% fetal bovine serum (FBS). The cell suspension was re-centrifuged at 470 x g for 5 min. The supernatant was discarded and the pellet was collected. The cell fraction was cultured overnight at 37°C in 5% CO2 in RCME containing 10% FBS. Non-adherent cells were removed after 24 h and the medium was changed to RKCM (K-STEMCELL media for MSC growth, K-STEMCELL) containing 5% FBS. The cells were maintained for 4-5 days until they reached confluence (passage 0). When the cells reached 90% confluence, they were expanded in RKCM until passage 3. Cell viability as evaluated by trypan blue exclusion before transplantation was greater than 95%. No evidence of bacterial, fungal or mycoplasma contamination was observed. Cell surface markers expressed by the AT-MSCs were analyzed using a FACS Caliber flow cytometer (BD Biosciences, San Jose, CA) with the CELL Quest software (Becton Dickinson, San Jose, CA). The AT-MSCs were positive for CD29, CD44, CD73, CD90, CD105 and HLA-ABC but were negative for CD31, CD34, CD45 and HLA-DR. AT-MSCs were used at passages 3–6 and sub-cultured once before use.

**Cancer cell culture.** A375SM, A375P (both human melanoma cell lines) and L929 (murine fibroblast cell line) cells were purchased from the Korea Cell Line Bank (Seoul, Korea). Melanoma cells were cultured in high-glucose Dulbecco’s modified Eagle’s medium (DMEM; Hyclone, Logan, UT, USA) containing 10% heat-inactivated FBS (Hyclone, Logan, UT), 1% penicillin and streptomycin (PS, Hyclone), 1.5 g/l sodium bicarbonate (Sigma-Aldrich, Strimheim, Germany) and 10 nM 4,(-2-hydroxyethyl)-1-piperazine ethanesulfonic acid (Hyclone). L929 cells were cultured in high-glucose DMEM containing 10% heat-inactivated FBS and 1% PS. Media supplementation or replenishment was carried-out every 2-3 days.

**Preparation of conditioned media.** Conditioned media derived from L929 cells and AT-MSCs were prepared as follows: 1x10^6 of each of the cells was cultured on 10-cm plates with 10 ml of a 1:1 mixture of DMEM and Ham’s F12 (DME/F12, Hyclone), 5% FBS and 1% PS for 48 h. The medium was harvested and filter-sterilized using a 0.22-μm Millex-HV syringe filter (Millipore, Billerica, MA, USA) and stored at ~80°C until use.

**In vitro cell viability assay.** The percentage of viable A375SM and A375P cells was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay. The human melanoma cells (2x10^4/well) were cultured in complete medium in 24-well plates for 24 h. The cells were cultured in triplicates with or without AT-MSC-CM and L929-conditioned medium (L929-CM) for another 72 h; the medium was replaced with fresh medium every 24 h. Control cells were cultured in DME/F12 1:1 medium supplemented with 5% FBS. Cell viability was measured by the MTT assay according to the manufacturer’s recommendations (Sigma, St. Louis, MO, USA). Briefly, 10 μl of MTT reagent (final concentration, 0.5 mg/ml) was added to the culture dishes and incubated for 2 h until a purple precipitate was visible. The supernatant was then aspirated and 100 μl of the detergent reagent was added. Absorbance at 540 nm was spectrophotometrically measured using a microplate ELISA reader (Bio-Rad, Hercules, CA, USA) with a reference wavelength of 630 nm. The results are expressed as the percentage of the values obtained in control conditions.

**Cell-cycle analysis.** For flow cytometry (FACS) analysis, A375SM and A375P cells (1x10^5 cells) were plated in 60-mm culture plates and cultured with AT-MSC-CM as described in the “in vitro cell viability assay” section. After 3 days, the cells were trypsinized, counted and fixed with 70% ethanol. For analysis of DNA content, the cells were labeled with propidium iodide (PI) (Sigma-Aldrich) in the presence of RNaseA (Sigma-Aldrich) (50 μg/ml, 30 min, 37°C in the dark). Samples were run on a FACScan flow cytometer (Becton-Dickinson, FL, NJ, USA) and data were analyzed using FCS Express 4 (De Novo Software, Thornhill, Ontario, Canada).

**Annexin V-FITC assay.** A375SM and A375P cells (1x10^5 cells) were plated in 60-mm culture plates and cultured with AT-MSC-CM for 72 h. The cells were then dissociated using trypsin–EDTA (Invitrogen) and washed with Annexin V binding buffer (1X). The cells were then labeled with either annexin-FITC (BioVision, Mountain View, CA, USA) or PI as per the manufacturer’s recommendations. Briefly, melanoma cells were collected by centrifugation and re-suspended in 500 μl of binding buffer. Then, 5 μl of Annexin V-FITC was added to the re-suspended cells. After incubation for 5 min on ice in the dark, 1 μg of PI was added to the cell suspension. Apoptotic and necrotic cells were quantified using a FACSscan flow cytometer and the Cell Quest pro software (Beckton-Dickinson).

**Western blot analysis.** For western blot analysis, A375SM and A375P cells were cultured with AT-MSC-CM for 72 h. Proteins were extracted from A375SM and A375P cells, resolved on SDS-polyacrylamide gels and transferred to a polyvinylidene fluoride (PVDF) membrane (Whatman, Maidstone, UK). The transferred membranes were blocked with 5% skim milk in Tris-buffered saline-Tween 20 (TBST: 0.1% Tween 20, 100 mM NaCl and 10 mM Tris-HCl, (pH 7.6)) for 2 h at room temperature. Blots were incubated with antibodies against cyclin D1, procaspase-3 and caspase-7 (1:250,
1:500 and 1:500 dilution, respectively) purchased from Santa Cruz Biotechnology Inc. (Heidelberg, Germany). Poly (ADP)-ribose polymerase (PARP) and β-actin antibodies (1:1000 and 1:5000 dilution, respectively) were purchased from Cell Signaling Technology (Ozyme, St Quentin en Yvelines, France). Secondary horse radish peroxidase (HRP)-conjugated antibodies (1:2000 dilution; Santa Cruz, Heidelberg, Germany) were added and blots were incubated in a blocking buffer for 2 h at room temperature. Immunoreactive proteins were visualized using the ECL plus kit (Gendepot, TX, USA).

**Scratch wound-induced migration assay.** Wound-induced migration assay was performed to assess the effect of AT-MSC-CM on melanoma cell migration as previously described (24). Briefly, melanoma cells were grown till they reached full confluence in 24-well plates coated with 2% gelatin and incubated overnight in starvation medium. Cell monolayers were wounded with a sterile 1-μl pipette tip and washed with phosphate-buffered saline (PBS) to remove the detached cells from the plates. Cells were either left untreated or treated with conditioned medium and kept in a CO₂ incubator for 64 h. The medium was replaced with fresh medium every 24 h. The wound gap was observed and cells were photographed using phase-contrast microscopy. The images were then analyzed using the Image J software 1.45s version (National Institutes of Health, USA) to measure the width of the scratch. The relative migration distance was calculated using the following formula: relative migration distance (%)=100 (a−b)/a, where a represents the width of the cell wounds before incubation and b represents the width of the cell wounds after incubation.

**Nude mouse transplantation.** Female, 6-week-old BALB/c nude mice were purchased from Central Lab. Animal, Inc. (Seoul, Republic of Korea). The mice were housed in a specific pathogen-free facility and allowed to acclimatize for 1 week to ensure that they were healthy before the start of the *in vivo* study. All animals were handled in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Seoul National University Institutional Animal Care and Use Committee. To induce human melanoma development in the mice, A375SM cells (5×10⁶ cells) suspended in PBS (100 μl) were injected subcutaneously (*s.c.* into the flanks of the mice. After 2 weeks, the mice that had developed tumors were randomly divided into two groups (n=5 for each group). Mice in the control group were administered a circumtumoral injection of PBS (100 μl). The mice in the AT-MSC group were treated with AT-MSCs (5×10⁶ cells) labeled with CM-DiI in PBS (100 μl); they were not given an intratumoral injection but an injection around the tumor site. PBS/AT-MSCs were injected five times every 3 days (on days 0, 3, 6, 9 and 12). Tumor size was measured every 3 days with a vernier caliper (Mitutoyo, Tokyo, Japan). Tumor volume was calculated using the following formula: tumor volume (mm³)=(a²×b)/2, where a and b represent the short and long axes respectively.

**Fluorescence staining analysis.** Fluorescence staining was performed to detect the presence of AT-MSCs at the tumor locus. Three days after the last injection of CM-DiI-labeled AT-MSCs, mice were sacrificed and tumor tissues were harvested. Tumor tissues were fixed in cold 4% paraformaldehyde for 4 h and then transferred to a sucrose medium (30% sucrose in 0.1 M PBS) for 16 h at 4°C. The tissues were then embedded in Tissue Tek OTC compound (Sakura Finetek, Torrance, CA, USA), snap-frozen in liquid nitrogen and stored at −80°C. The frozen tissue was sectioned (15-μm-thick sections) and mounted on slides. The nuclei of all the cells were stained with Hoechst 33342 (10 μg/ml) for 30 min in the dark. Images were captured with a confocal microscope (Nikon, Eclipse TE2000, Tokyo, Japan) and processed using the Image J software 1.45s version.

**Statistical analysis.** All experimental data were analyzed using the GraphPad Prism (version 4) software (Graphpad Software Inc., San Diego, CA, USA). All data are presented as mean±standard deviation (SD). The statistical significance of mean values in multiple sample groups was examined using Bonferroni’s comparison test after one-way ANOVA. Statistical differences between the mean values of the two sample groups were determined using the Student’s *t*-test. *p*-Values <0.05 were considered to indicate statistical significance.

**Results**

**In vitro inhibition of melanoma cell growth by AT-MSC-CM.** AT-MSC-CM significantly inhibited the viability of A375SM (75.09% vs. control cells, *p*<0.001) and A375P (75.41% vs. control cells, *p*<0.001) cells (Figure 1). Because the observed responses may reflect the exhaustion of nutrients from media or the non-specific accumulation of toxic metabolites, conditioned medium from L929 fibroblast cells was used as a control, together with non-conditioned medium. In contrast to AT-MSC-CM, treatment with L929-CM did not impair melanoma cell viability. These results show that AT-MSC-CM has *in vitro* anti-proliferative effects against human melanoma cells.

**Effect of AT-MSC-CM on the cell cycle of melanoma cells.** The cell cycle status of A375SM and A375P cells was analyzed 72 h after the cells were cultured with AT-MSC-CM (Figure 2). The amount of melanoma cells cultured with AT-MSC-CM showed an increase in the G0/G1 phase of the cell cycle compared to controls, which suggests that more melanoma cells are arrested at the G0/G1 phase in the presence of AT-MSC-CM. The proportion of cells in the G0/G1 phase was 65.20% and 50.73% for A375SM cells treated with and without AT-MSC-CM respectively (*p*<0.001) (Figure 2A and 2B); furthermore, it was 72.63% and 61.18% for A375P cells treated with and without AT-MSC-CM respectively (*p*<0.0001) (Figure 2C and 2D). This increase was coupled with a decreased percentage of tumor cells in the S phase. The percentages of A375SM and A375P cells cultured with AT-MSC-CM in the S phase was 19.08% and 18.89%, respectively, whereas it was 32.44% and 28.99%, respectively, for A375SM and A375P cells cultured in control medium (*p*<0.0001 and *p*<0.0001 for A375SM and A375P cells, respectively). We next measured the expression of the main proteins involved in cell-cycle regulation. The expression of cyclin D1 in melanoma cells cultured with AT-MSC-CM for 72 h was decreased (Figure 2E). These results indicate that AT-MSC-CM induces G0/G1 cell-cycle arrest of melanoma cells by down-regulation of cyclin D1 expression.
Apoptotic effect of AT-MSC-CM on melanoma cells. A375SM (Figure 3A) and A375P (Figure 3B) cells were analyzed by flow cytometry with Annexin V/PI staining after culture with AT-MSC-CM for 72 h. As shown in Figure 3C, the apoptosis rates were 5.66% and 5.52% for A375SM and A375P cells, respectively; for the control A375SM and A375P cells, the apoptosis rates were 3.85% and 4.52%, respectively. The apoptosis rates of cells treated with AT-MSC-CM were significantly different from those of the control cells (p<0.01 for both A375AM and A375P cells). The effects of AT-MSC-CM on procaspase-3, caspase-7 and PARP levels were analyzed by western blotting to elucidate the underlying biochemical mechanisms involved in the regulation of apoptosis. Procaspase-3 expression in melanoma cells cultured with AT-MSC-CM was down-regulated, whereas cleaved PARP and caspase-7 expression showed a marked increase. These findings show that AT-MSC-CM can induce melanoma cell apoptosis through caspase-3/7 and PARP activation.

Effect of AT-MSC-CM on human melanoma cell migration. The wound-induced migration assay showed that the major part of the gap or wounding space between cell layers after wounding was occupied by migrating melanoma cells that were treated with the control medium and L929-CM (Figure 4A and 4B). However, the healing of the wound or the empty space in the cells was largely not occupied by migrating cells treated with AT-MSC-CM. As shown in Figure 4C, relative to L929-CM-treated and untreated control cells, AT-MSC-CM-treated A375SM, as well as A375P cells, showed reduced migration capacity. These findings suggest that AT-MSC-CM inhibited migration of melanoma cells.

Melanoma suppression by AT-MSCs in mouse xenograft model. To evaluate AT-MSC-dependent growth inhibition of human melanoma, a xenograft study of A375SM cells was carried out using female athymic mice. Fourteen days after tumor engraftment, mice were given circumtumoral AT-MSCs or PBS (control). The control tumors grew rapidly and their average size was 2,907.5±843.5 mm$^3$ within 27 days following transplantation of AT-MSCs (Figure 5A and 5B). In contrast, the tumor volumes were significantly reduced in the AT-MSC group (1,496.8±434.4 mm$^3$, p<0.05). To confirm the migration of transplanted AT-MSCs to melanoma cells in the A375SM cell xenograft model, AT-MSCs were labeled with the cell tracker dye CM-DiI before in vivo administration. Three days after the last administration of AT-MSCs in mice, the tissue was harvested and frozen tumor sections were made. The Hoechst 33342-stained cell nuclei appeared blue and the CM-DiI-labeled AT-MSCs were red in the confocal fluorescence micrograph (Figure 6). These results suggest that circumtumorially-injected AT-MSCs effectively migrate to the tumor region.

Discussion

Cancer remains one of the major causes of mortality and morbidity throughout the world. The common conventional approaches of surgery, chemotherapy and radiotherapy are often limited by the recurrence of metastasis or therapy-related life-threatening complications (25). Despite development of various tumor-targeted therapeutic methods, the overall outcome of cancer patients has not remarkably improved. For these reasons, there is an urgent need for alternative therapeutic strategies that specifically target malignant cells. MSCs are a population of adult stem cells with the potential for self-renewal and differentiation into multiple cell types. There is ample evidence that AT-MSCs and other MSC types can inhibit tumor growth in vitro and in vivo. For example, human BM-MSCs have been shown to inhibit tumor growth in immunodeficient mice bearing disseminated non-Hodgkin’s lymphoma xenografts (26). Furthermore, another study demonstrated that rat umbilical cord matrix stem (rUCMS) cells completely attenuated rat mammary adenocarcinoma with no evidence of metastasis or recurrence after the tumor was inoculated with rUCMS cells (27). Anti-tumor effects were also demonstrated in MSCs harvested from adipose tissues. Cousin et al. showed that a single intra-tumoral injection of AT-MSCs in a model of pancreatic adenocarcinoma inhibited tumor growth (28). More recently, AT-MSC-CM was shown to significantly inhibit the growth of human U251 glioma cells in vitro (29). Yang et al. also found...
that growth of several other tumor cell lines, including the rectal cancer cell line HT29, lung cancer cell line A549 and breast cancer cell line MCF-7, was inhibited by AT-MSC-CM (29). However, there are several critical questions to be addressed in order to develop effective treatments for cancer therapy. In contrast to the findings mentioned above, some studies have shown that MSCs resulted in systemic immunosuppression that favored tumor growth in vivo (30). For example, Sun et al. found that BM-MSCs played an important role in tumor angiogenesis and promoted proliferation of melanoma cells (22). Although MSCs have received attention for their potential use in clinical therapy, there are still several unsolved problems that limit the application of MSCs.

The present study aimed to evaluate the effect of AT-MSCs on melanoma growth in two experimental systems and the mechanisms by which AT-MSCs exert their actions. First, we investigated the effect of AT-MSCs on tumor cell proliferation by treatment of tumor cells with AT-MSC-CM. Our results clearly showed that AT-MSC-CM can inhibit the proliferation of A375SM and A375P melanoma cells in vitro. Khakoo et al. reported that MSCs inhibit the growth of Kaposi’s sarcoma by cell-to-cell contact (31). However, the present study found that proliferation of melanoma cells was inhibited after melanoma cells were treated with AT-MSC-CM, which indicates that certain soluble factors secreted by AT-MSCs may inhibit melanoma cell proliferation without cell-to-cell contact. In support of this, it has been reported that the regulatory elements in cell-conditioned medium may influence various signaling mechanisms, such as transcription initiation, differential gene expression and re-programming of specific cell types (29). However, the putative molecules in the conditioned medium responsible for altering the cell fate still remain unclear and require further research.

Tumorigenesis is the result of cell cycle disturbance, which leads to uncontrolled cellular proliferation. Cell proliferation and differentiation are specifically controlled in the G1 phase and the G1/S phase transition in the cell cycle (32). In our study, a higher amount of A375SM and A375P cells treated with AT-MSC-CM were found in the G0/G1 phase compared to controls. Therefore, we have reason to believe that AT-MSC-CM represses cell growth via cell cycle arrest in the G0/G1 phase.
phase. In this report, cyclin D1 levels decreased in A375SM and A375P cells treated with AT-MSC-CM, which probably indicates that AT-MSC-CM can down-regulate the cyclin D1 protein level, ultimately leading to cell cycle arrest of melanoma cells. The main families of regulatory proteins that play major roles in controlling cell-cycle progression are the cyclin-dependent kinases (Cdks), cyclins, the Cdk inhibitors (CKI) and the tumor-suppressor gene products p53 and pRb (33). It is, therefore, possible that AT-MSC-CM disrupts these cell cycle regulatory mechanisms in A375SM and A375P cells.

To determine the mechanism by which AT-MSC-CM inhibited melanoma cell proliferation, we performed Annexin V/PI flow cytometric assays. The flow cytometric assay indicated that AT-MSC-CM can induce apoptosis in A375SM and A375P cell lines. Caspases play a key role in various forms of apoptosis (34). It is widely believed that activation of caspase-3/7 leads to DNA fragmentation, a hallmark of apoptosis. In the present report, the procaspase-3 levels decreased and caspase-7 levels increased in A375SM and A375P cells treated with AT-MSC-CM. We, therefore, assumed that up-regulation of cleaved caspase-3 leads to down-regulation of procaspase-3. PARP is part of a family of proteins involved in a number of cellular processes that play a role in DNA repair, DNA stability and programmed cell death (35). It has been reported that caspase-3 and caspase-7 are the most efficient proteases for PARP cleavage (36). In the present study, cleaved PARP was up-regulated in A375SM and A375P cells treated with AT-MSC-CM. These findings indicate that AT-MSC-CM can trigger caspase-3/7 activation and, thus, PARP cleavage in melanoma cells ultimately leading to apoptosis. Our results are also consistent with the findings of Takahara et al., according to which AT-MSCs induced apoptosis of prostate cancer cells by activating the caspase 3/7 signaling pathway (37). They have shown that the anti-proliferative effect of AT-MSCs on prostate cancer cells appears to be mediated by TGF-β1 secretion and signaling.

In our study, AT-MSC-CM was found to inhibit the movement of A375SM and A375P melanoma cells. These observations suggest that AT-MSCs secrete a chemical mediator that inhibits the migratory capacity of melanoma cells. Although we could not determine the exact mechanism, our findings are in agreement with recent reports showing that MSCs inhibit the
migration and invasion of cancer cells (38-40). TIMP-1, TIMP-2 and inhibitors of matrix metalloproteinases (MMPs) were identified as candidates for this inhibition (38). Another study demonstrated that the inhibitory effect on tumor migration was mediated by up-regulation of phosphatase and tensin homolog (PTEN) in glioma cells by cord blood MSCs (40).

To evaluate the therapeutic effects of AT-MSCs \textit{in vivo}, a melanoma xenograft model was created by injecting human melanoma A375SM cells into flanks of mice. When AT-MSCs were administered circumtumorally in tumor-bearing nude mice, tumor growth was inhibited. The homing of MSCs to tumors is well-established (41). In that study too, we administered AT-MSCs circumtumorally and observed that they could find their way into tumors. This homing ability of MSCs seems to be mediated by chemokines and growth factors secreted by the tumors or their associated stroma (14, 25). The homing ability of MSCs has previously been exploited for drug delivery and targeted gene delivery (12, 13, 42, 43). The ability of unengineered AT-MSCs to inhibit melanomas is a distinct advantage because any manipulation causing the cells to express an exogenous gene could alter them in some way that would potentially make them less safe as transplantable cells.

In conclusion, we provided evidence showing that AT-MSC-CM has an anti-proliferative effect on melanoma cells, which is brought about \textit{via} cell cycle arrest and apoptosis of tumor cells, and that AT-MSCs have an inhibitory effect on the growth of A375SM cell-derived tumors \textit{in vivo}. Since AT-MSCs are easily obtained without any ethical concerns, cell therapy using AT-MSCs appears promising as a therapeutic option for melanoma, although further research on the clinical application of AT-MSCs is needed.

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Figure 5. The effect of AT-MSCs on A375SM melanoma growth in BALB/c nude mice. A total of 5.0×10^6 A375SM cells were inoculated subcutaneously into the flank of each mouse and AT-MSCs were injected circumtumorally in the treatment group. The size of each tumor mass was measured every 3 days with a Vernier caliper. (A) Representative tumors on day 15 in athymic nude mice. (B) Tumor volumes were significantly reduced in the AT-MSC treatment group in comparison to mice that received PBS as a control. Values were expressed as means±SD and determination of statistical significance was performed using a Student’s t-test (*p<0.05).

Figure 6. Fluorescence images of AT-MSCs in melanoma tumor locus. AT-MSCs were labeled with CM-DiI and injected into tumor-bearing nude mice. Circumtumorally administered CM-DiI labeled AT-MSCs integrated into tumor locus. Sections were counterstained with Hoechst 33342 nuclear staining (blue), CM-DiI labeled AT-MSCs (red) and A375SM melanoma tumor cells identified by confocal fluorescence microscopy of tumor sections. Magnification ×200.
References


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