Abstract. Background/Aim: Human mesenchymal stem cells (hMSCs) are thought to be one of the most reliable stem cell sources for a variety of cell therapies. This study investigated the anti-tumor effect of human adipose tissue-derived mesenchymal stem cells (hAT-MSCs) on EL4 murine T-cell lymphoma in vitro and in vivo. Materials and Methods: The growth-inhibitory effect of hAT-MSCs on EL4 tumor cells was evaluated using a WST-1 cell proliferation assay. Cell-cycle arrest and apoptosis were investigated by flow cytometry and western blot. To evaluate an anti-tumor effect of hAT-MSCs on T-cell lymphoma in vivo, CM-DiI-labeled hAT-MSCs were circumtumorally injected in tumor-bearing nude mice, and tumor size was measured. Results: hAT-MSCs inhibited T-cell lymphoma growth by altering cell-cycle progression and inducing apoptosis in vitro. hAT-MSCs inhibited tumor growth in tumor-bearing nude mice and prolonged survival time. Immunofluorescence analysis showed that hAT-MSCs migrated to tumor sites. Conclusion: hAT-MSCs suppress the growth of T-cell lymphoma, suggesting a therapeutic option for T-cell lymphoma.

T-cell lymphoma is a heterogeneous group of lymphoid neoplasms derived from a T-cell lineage. T-cell neoplasms are divided into precursor T-cell neoplasms and peripheral T-cell neoplasms (1, 2). Among the peripheral T-cell neoplasms, primary extranodal disease and predominantly nodal disease are referred to as peripheral T-cell lymphoma.

Peripheral T-cell lymphomas account for approximately 25% of all non-Hodgkin’s lymphomas in Korea and 12-15% of all cases in the North American Caucasian population (2, 3). The prognosis is guarded to poor (5-year survival time: 25%) for many peripheral T-cell lymphomas and current treatment strategies are largely ineffective; thus a novel approach is required (3, 4).

Mesenchymal stem cells (MSCs) or multipotent mesenchymal stromal cells are a heterogeneous population of cells that proliferate in vitro as plastic-adherent cells, have fibroblast-like morphology, form colonies in vitro and can differentiate into bone, cartilage and fat cells. MSCs have been successfully isolated from nearly every organ and many tissues including: brain, liver, kidney, lung, bone marrow, muscle, thymus, pancreas, skin, aorta, vena cava, adipose tissue, fetal tissue, umbilical cord, Wharton’s jelly and placenta (5-7). Adipose tissue obtained from subcutaneous tissue represents the most plentiful potential source for harvesting MSCs reliably using a less invasive procedure than that required for other types of MSCs (8). These characteristics make them an attractive source for clinical and research use.

MSCs are considered to be promising candidates for clinical application due to the ease of isolation from adult donors, thus obviating the ethical concerns concerning embryonic stem cell research (9). MSCs potently modulate immune responses and have paracrine effects through secretion of growth factors, cytokines and anti-fibrotic or angiogenic mediators (10). Thus, MSCs have a broad prospect of clinical application in regenerative medicine and cancer therapy. MSCs secrete a variety of cytokines and molecules that are known to influence tumor proliferation, migration and angiogenesis (11-14). MSCs may also act directly by intercellular signaling via cell-to-cell contacts with neighboring cells, such as tumor cells, stromal fibroblasts, infiltrating immune/inflammatory cells and lymphatic and blood vascular networks, that ultimately determine the fate of tumor growth kinetics (14). Several

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studies suggest that MSCs migrate to the tumor site where they contribute to tumor reduction (11, 15, 16). The ability of MSCs to specifically migrate to tumors suggests a potential use as a vehicle for the delivery of therapeutic genes to tumors. However, given the numerous conflicting and controversial studies on the ability of MSCs to support tumor growth, it is important to further investigate the role of MSCs in the tumor microenvironment (17, 18).

In the present study, human adipose tissue-derived mesenchymal stem cells (hAT-MSCs) were transplanted into a mouse T-cell lymphoma model to determine the therapeutic potential of human AT-MSCs on T-cell lymphoma. The results indicate that hAT-MSCs inhibit the growth of T-cell lymphoma by inducing cell-cycle arrest and apoptosis.

Materials and Methods

Cell culture. The EL4 (murine T-cell lymphoma) cell line was purchased from the Korean Cell Line Bank (Seoul, South Korea). Lymphoma cells were cultured in high-glucose Dulbecco’s Modified Eagle’s Medium (DMEM; Hyclone, Logan, UT, USA) containing 10% heat-inactivated fetal bovine serum (FBS; Hyclone), 1% penicillin and streptomycin (PS; Hyclone), 1.5 g/l sodium bicarbonate (Sigma-Aldrich, Steinheim, Germany) and 10 mM 4-[2-hydroxyethyl]-1-piperazine ethanesulphonic acid (HEPES; Hyclone). The cell culture medium was changed every 2-3 days. Human adipose tissue samples were obtained by simple liposuction of abdominal subcutaneous fat from donors after informed consent. hAT-MSCs were prepared under Good Manufacturing Practice conditions (K-STEMCELL CO., Ltd, Seoul, South Korea), as described previously (19). Briefly, subcutaneous adipose tissues were digested with 4 ml of RTase cell isolation enzyme (K-STEMCELL) per gram of fat 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 collected by centrifugation at 1,500 rpm for 5 min. The cell pellet was resuspended in RCME (K-STEMCELL media for MSC attachment, K-STEMCELL) containing 10% FBS. After another centrifugation at 1500 rpm for 5 min, the supernatant was discarded and cells in the pellet were cultured overnight at 37˚C in 5% CO2 in RCME containing 10% FBS. Cell adhesion was examined under an inverted microscope 24 h later. Non-adherent cells were removed after 24 h, adherent cells were washed with PBS and the medium changed to RKCM containing 5% FBS. The cells were maintained for 4-5 days until confluent (passage 0) and then expanded in RKCM until passage 3 at 90% confluence. The immunophenotypes of the hAT-MSCs were analysed using a FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) and CellQuest software (Becton Dickinson, San Jose, CA). Every harvest of hAT-MSCs revealed a homogeneous population of cells with the characteristics of mesenchymal stem cells. The cells expressed mesenchymal stem cell markers (CD90, CD105, CD44 and CD29) and did not express hematopoietic or endothelial markers (CD31, CD34 and CD45). Cell viability evaluated by trypan blue exclusion before transplantation was greater than 95%. No evidence of bacterial, fungal or mycoplasma contamination was observed. hAT-MSCs were used between passages 3-6 and subcultured once before use. All cells were cultured at 37˚C in a 5% CO2 incubator.

Cell proliferation assay. hAT-MSCs (5.0×10³ cells/well) were plated in a 96-well plate for direct co-culture with EL4 cells. After confirming the attachment of hAT-MSCs, adherent cells were thoroughly washed twice with PBS after 24 h and seeded with EL4 cells (5.0×10³ cells). Cell proliferation assays were performed after 1, 2 or 3 days of incubation, respectively, using the WST-1 cell proliferation assay system kit (Takara...
Bio Inc., Otsu, Shiga, Japan) according to the manufacturer’s instructions. Briefly, WST-1 cell proliferation reagent was added to 10% of the total medium volume. Cells were incubated for 2 h in a humidified atmosphere (37°C, 5% CO2). After incubation, absorbances were measured at a wavelength of 450 nm using a microplate ELISA reader (BioRad Laboratories Inc., Hercules, CA, USA).

In an indirect co-culture system, hAT-MSCs (7.5×10^3 cells/well) were plated in a 24-well plate (0.4 μm PE membrane, SPL Life Sciences, Co. Ltd. Gyeonggi-do, South Korea); after 24 h, EL4 lymphoma cells (7.5×10^3 cells) were seeded into cell culture inserts placed in the wells. After 1, 2 or 3 days of incubation, cell proliferation was measured as above.

Figure 2. Cell cycle arrest of EL4 co-cultured with hAT-MSCs. For analysis of the cell cycle distribution of tumor cells, hAT-MSCs (2.0×10^5 cells/well) were cultured in a 6-well plate. After 24 h, EL4 cells (2.0×10^5 cells/well) were seeded. (A) Cell cycle progression analyzed via DNA histogram after culturing EL4 T-cell lymphoma cells with or without hAT-MSCs for 1 day. (B) EL4 cells co-cultured with hAT-MSCs showed an increase in the G0/G1 phase of the cell cycle compared to controls (p<0.001). G1 arrest occurred concurrently with a reduction in the percentage of S phase cells (p<0.01). The p-value was obtained using Student’s t test by comparing treated hAT-MSCs with control (EL4 alone) for each phase of the cell cycle. All experiments were independently conducted in triplicate and values expressed as the mean±SD. **p<0.01, ***p<0.001.

Figure 3. The apoptotic effect of hAT-MSCs on EL4 T-cell lymphoma cells. For the analysis of annexinV/PI and cell cycle distribution of tumor cells, hAT-MSCs (2.0×10^5 cells/well) were cultured in a 6-well plate. After 24 h, EL4 cells (2.0×10^5 cells) were seeded. (A) EL4 T-cell lymphoma cells in 6-well plates were cultured for 1 or 3 days, with or without hAT-MSCs, and cell apoptosis was evaluated by flow cytometry using Annexin V and PI staining. EL4 cells treated with hAT-MSCs demonstrated a higher apoptosis ratio than those of the control. All experiments were independently conducted in triplicate and values expressed as the mean±SD. (B) Western blot showing the expression of procaspase-3 and cleaved PARP proteins in EL4 cells cultured with hAT-MSCs. Samples were standardized according to β-actin protein levels.
Annexin V-FITC assay.

Thornhill, Ontario, Canada). NJ, USA) and data analyzed by FCS Express 4 (De Novo Software, FACScan flow cytometer (Becton-Dickinson Corp., Franklin Lakes, New Jersey, USA; Annexin V-FITC Apoptosis Detection kit). Brieﬂy, EL4 cells were collected by centrifugation, resuspended in 500 μl of cold 4% paraformaldehyde. Tumor tissue from each group of mice was harvested and ﬁxed in 70% pre-chilled ethanol overnight. The next day, cells were treated according to the manufacturer’s instructions (BioVision, Mountain View, CA, USA; Annexin V-FITC Apoptosis Detection kit).

Cell-cycle analysis. hAT-MSCs (2.0×10^5 cells/well) were plated in a 6-well plate and co-incubated with 2.0×10^5 EL4 cells after 24 h. After one day, EL4 cells were collected by centrifugation, counted and ﬁxed in 70% pre-chilled ethanol overnight. The next day, cells were incubated in PBS containing propidium iodide (PI; 50 μg/ml) and RNase A (50 μg/ml) for 1 h at room temperature. The ﬂuorescence of 10,000 cells per sample was measured using a FACScan ﬂow cytometer (Becton-Dickinson Corp., Franklin Lakes, NJ, USA) and data analyzed by FCS Express 4 (De Novo Software, Thornhill, Ontario, Canada).

Annexin V-FITC assay. To determine hAT-MSCs-induced apoptotic cell death of EL4 tumor cells, hAT-MSCs (2.0×10^5 cells/well) were plated in a 6-well plate and co-incubated with 2.0×10^5 EL4 cells after 24 h. After cells were co-cultured for 1 or 3 days, respectively, cells were treated according to the manufacturer’s instructions (BioVision, Mountain View, CA, USA; Annexin V-FITC Apoptosis Detection kit). Brieﬂy, EL4 cells were collected by centrifugation, resuspended in 500 μl of binding buffer and 5 μl of Annexin V-FITC added. After incubation for 5 min on ice in the dark, 1 μg of PI was added to the cell suspension. The samples were then analysed by generating a plot showing FL1 (for Annexin V-FITC) using the ﬂow cytometer.

Western blot. Procaspase-3, poly ADP-ribose polymerase (PARP) and β-actin expression levels were examined in EL4 cells by western blot. EL4 cells and hAT-MSCs were co-cultured for 1 or 3 days, respectively, and then EL4 cells collected at regular time intervals. Cells (5×10^6 cells) were lysed in 200 μl of cold protein lysis buffer (Intron Co. Ltd., Seoul, Korea) for 1 h and kept on ice to stop further biochemical activity. Protein content was quantiﬁed according to the Bradford method before adding sample buffer. Equal amounts of protein samples (60 μg/sample) were loaded on SDS-PAGE gels and transferred to PVDF membranes (Whatman Inc., Sanford, ME, USA). The membranes were blocked with Tris-buffered saline-Tween 20 (TBST, 0.1% Tween 20/100 mM NaCl/10 mM Tris-HCl, pH 7.6) containing 5% non-fat dried milk for at least 1 h and then the blots were probed with procaspase-3, PARP and β-actin antibodies (1:200, 1:500 and 1:1000 dilution, respectively) at 4°C overnight. The membranes were washed three times and then incubated with horseradish peroxidase-conjugated anti-IgG (1:2000 dilution) in a blocking buffer for 2 h. Finally, the blots were developed with enhanced chemiluminescence (Gen Depot, TX, USA) and exposed to X-ray ﬁlm (Eastman-Kodak, Rochester, NY, USA). Polyclonal anti-PARP was purchased from Cell Signaling Technology (#9542; Danvers, MA, USA). Anti-caspase-3 (H-277) and anti-β-actin (sc-47778) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Effect of hAT-MSCs on growth of EL4 in vivo. Animals were purchased from Central Lab. Animal, Inc. (Seoul, Republic of Korea). Female BALB/c nude mice (20-30 g) aged 6-8 weeks were used in experiments. Animals were housed in micro-isolator cages under clean conditions and observed for at least one week to ensure they were healthy prior to commencing in vivo studies. Lighting, temperature, and humidity were controlled automatically. Food and water were provided ad libitum. All animals were handled in strict accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Protocols were approved by the Seoul National University Institutional Animal Care and Use Committee (Permit Number: SNU-140418-7).

To induce mouse T-lymphoma development in animals, EL4 cells (5.0×10^5) suspended in 100 μl PBS were injected subcutaneously into the right flank of each mouse. After the development of 5 to 6 mm diameter-sized tumors, mice were randomly assigned to two groups (n=6/group): group 1 was given circumtumoral PBS as a control and group 2 was given circumtumoral hAT-MSCs (5.0×10^5/100 μl PBS) five times every 3 days (on days 0, 3, 6, 9 and 12). The size of each tumor mass was measured every 3 days with a vernier caliper (Mitutoyo, Tokyo, Japan). Tumor volume was calculated using the following formula: tumor volume (mm^3)=(a^2 × b)/2, where ‘a’ is the length of the short axis and ‘b’ is the length of the long axis. Survival was calculated as the duration of the animal’s life span from the time of inoculation of lymphoma cells until death.

Tissue processing and imaging of transplanted hAT-MSCs. Migration of hAT-MSCs to tumor tissue in vivo was determined by confocal microscopy analysis of cells labelled with the red ﬂuorescent dye, CM-DiI (Invitrogen, Carlsbad, CA) as follows: The CM-DiI stock solution of lipophilic tracer was prepared in dimethyl sulfoxide at 1 mg/ml. After the hAT-MSCs were washed twice with PBS, the cells were resuspended in 2 ml of Dulbecco’s phosphate buffered saline (DPBS) and 5 μl CM-DiI solution was added. Cells were incubated for 5 min at 37°C, then for an additional 15 min at 4°C. After labelling, cells were washed with PBS and CM-DiI labeled hAT-MSCs injected circumtumorially into mice exhibiting tumor formation. Three days after the last injection, mice were sacriﬁced and tumor tissue was harvested and ﬁxed in 4% parafomaldehyde. Tumor tissue from each group of mice was embedded in Tissue Tek OTC compound (Sakura Finetek, CA, USA), snap-frozen in liquid nitrogen and stored at ~80°C. Frozen tissue was sectioned (15 μm-thick sections), mounted onto slides and stained with Hoechst 33342 (10 μg/ml) for 30 min in the dark.
to visualize cell nuclei. Images were captured with a confocal microscope (Nikon, Eclipse TE200, Japan) and processed using the Image J software 1.45s version (National Institutes of Health, USA).

Statistical analysis. All experimental data were expressed as mean±standard deviation (SD). Statistically significant differences were assessed by the Student’s t-test using the GraphPad Prism (version 4) software (Graphpad Software Inc., San Diego, CA, USA). Analysis of survival data was carried out using the GraphPad prism software; in particular, differences in survival between treatment groups were calculated using the Kaplan-Meier curve and the survival distributions of the treated and control groups were compared using the log-rank test. Differences between two conditions at p<0.05 were considered statistically significant.

Results

hAT-MSCs inhibit the growth of EL4 in vitro. To investigate whether hAT-MSCs have an inhibitory effect on lymphoma cell growth and viability in vitro, EL4 cells were co-cultured with hAT-MSCs at the same ratio in a direct co-culture system. Proliferation of EL4 cells was significantly reduced by day 3 for the hAT-MSCs treatment group compared to the untreated control group (78.77% of control growth, p<0.01, Figure 1A). When hAT-MSCs and EL4 cells were co-cultured but separated by a porous membrane which allowed the exchange of soluble factors without cell-to-cell contact, EL4 proliferation was also significantly reduced (71.05% of control growth, p<0.001, Figure 1B). These results show that hAT-MSCs have an anti-proliferative effect on T-cell lymphoma cells in vitro.

hAT-MSCs induce cell cycle arrest in EL4 cells. EL4 T-cell lymphoma cells were co-cultured with hAT-MSCs and tumor cells harvested for cell-cycle distribution analysis. EL4 cells co-cultured with hAT-MSCs showed increases in the G0/G1 phase of the cell cycle compared to controls (p<0.001, Figures 2A and 2B). The proportions of EL4 cells in the G0/G1 phase treated with or without hAT-MSCs were 53.15% and 47.69%, respectively. This increase of tumor cells in the G0/G1 phase with hAT-MSCs co-culture was 53.15% and 47.69%, respectively. This increase of tumor cells in the G0/G1 phase with hAT-MSCs co-culture was 53.15% and 47.69%, respectively.

hAT-MSCs induce apoptosis in EL4 cells. To investigate whether hAT-MSCs inhibit tumor cell proliferation by inducing apoptosis, EL4 T-cell lymphoma cells were analyzed by flow cytometry with Annexin V/PI staining after co-culture with hAT-MSCs for 1 or 3 days. An increase in EL4 cell apoptosis was observed after 3 days of hAT-MSCs co-culture. As shown in Figure 3A, the Annexin V population increased from 4.7% to 7.6% (p<0.01, day 3) compared to that of the control group not cultured with hAT-MSCs. These results indicate that EL4 cell apoptosis is induced by hAT-MSCs. The effect of hAT-MSCs on caspase-3 and PARP protein levels was analyzed by western blotting to elucidate the underlying biochemical mechanisms involved in the regulation of apoptosis. As shown in Figure 3B, procaspase-3 protein expression was down-regulated in EL4 cells co-cultured with hAT-MSCs for 3 days, whereas cleaved PARP protein expression increased markedly. These observations demonstrate that hAT-MSCs can induce EL4 cell apoptosis through caspase-3 and PARP activation.

Injection of hAT-MSCs inhibits tumor growth in an EL4 mouse T lymphoma model. To evaluate the effect of hAT-MSCs on tumor growth in vivo, an EL4 T-cell lymphoma model was established in female BALB/c nude mice. When tumor volumes reached a mean value of 378 ± 101 mm³ six days after subcutaneous injection of EL4 cells, mice were injected with circumtumoral hAT-MSCs or PBS as a control. As shown in Figure 4, tumor volume was significantly reduced in the hAT-MSCs treatment group. Moreover, improved survival was paralleled by a delay in tumor growth in mice injected with hAT-MSCs compared to mice injected with PBS (Figure 5).

hAT-MSCs migrate to the tumor region. To track the migration of transplanted hAT-MSCs to tumors in the EL4 T-cell lymphoma model, hAT-MSCs were labeled with the cell tracker dye CM-DiI before in vivo administration. Three days after the last administration of hAT-MSCs to mice, tissue was harvested and frozen tumor sections prepared. Immunofluorescence analysis demonstrated the presence of hAT-MSCs in the tumor region, indicating circumtumorally injected hAT-MSCs effectively migrated to the intratumoral region from the outer layer of the tumor (Figure 6).
Discussion

MSCs show promising potential for use in regenerative medicine and a variety of diseases because of their self-renewal and multipotential differentiation abilities. In addition to recent studies describing their tissue regeneration capability, MSCs have been identified as promising therapeutic tools in cancer treatment (11, 20-22). MSC-based anticancer cell therapy relies on predominantly tumor-specific selections provided by MSCs and MSC-carried anticancer agents. MSCs possess a powerful capacity for tumor-directed migration and incorporation, highlighting their potential as optimal vehicles for delivering anticancer agents (21, 22). Homing in directly to the tumor...

Figure 6. Fluorescence images of hAT-MSCs localized to T-cell lymphoma tumors. hAT-MSCs were labeled with CM-Dil and injected into tumor-bearing animals. Subcutaneously administered CM-Dil labeled hAT-MSCs localized to tumors. Sections were counterstained with Hoechst 33342 nuclear stain (blue). CM-Dil labeled hAT-MSCs (red) and T-cell lymphoma tumor cells identified by confocal fluorescence microscopy of tumor sections. Magnification ×200.
microenvironment, engineered MSCs are able to express and/or release anticancer agents that constantly act on adjacent tumor cells. However, there are several critical questions that need to be addressed in order to develop effective treatments for cancer therapy. Many studies have reported contradicting results: some investigators have discovered that MSCs promote tumor growth and others report that MSCs inhibit tumor growth (23, 24). The anti-tumor effects of MSCs are highly variable depending on the type of MSCs and tumor cells. Thus, the effect of MSCs on the proliferation of the targeted tumor should be investigated prior to the use of MSCs as a tool for drug delivery. The present study demonstrates an anti-tumor effect of hAT-MSCs on T-cell lymphoma both in vitro and in vivo.

In particular, this study demonstrates that hAT-MSCs inhibited T-cell lymphoma cell growth both in direct and indirect co-culture in vitro. These results indicate that hATMSCs have both direct and indirect anti-proliferative effects on T-cell lymphoma cells. Previously, different mechanisms underlying the antiproliferative effects of MSCs on tumor cells have been proposed. A previous study demonstrated that bone marrow-derived mesenchymal stem cells (BM-MSCs) exerted a potent anti-oncogenic effect on Kaposi’s sarcoma through cell-to-cell contact and Akt inactivation (11). Another study provided an approach to specifically examine the effect of the stem cell microenvironment on cancer cells without the complexity of cell-to-cell interactions (25). It has been reported that MSCs inhibited angiogenesis and suppressed tumor growth in human lymphoma and glioma (14, 26). Our data demonstrate that hAT-MSCs have the ability to inhibit the proliferation of tumor cells by altering cell cycle progression (Figure 2). EL4 lymphoma cells co-cultured with hAT-MSCs exhibited a predominantly G0/G1 phase accumulation predominantly and a decrease in S phase compared to controls. BM-MSCs have a potentially inhibitory effect on the growth of other lymphoma cell lines (i.e. YAC-1) in vitro by inducing G0/G1 phase arrest rather than inducing apoptotic cell death (27). Another study has demonstrated that the inhibitory effect on tumor growth was mediated by an inhibitor of the β-catenin pathway, DKK-1, which is secreted by MSCs (12, 28). Tumor inhibition may thus be induced by down-regulation of positive cell cycle regulators, such as cyclin D1, D2 and CDK4, along with up-regulation of the negative regulator, cyclin dependent kinase inhibitor, p27, and its subsequent inhibition of Rb phosphorylation and G1 arrest (29).

To further explore the mechanism by which hAT-MSCs inhibited EL4 cell proliferation, EL4 cell death was measured by Annexin V staining and flow cytometry. The result revealed that EL4 cells treated with hAT-MSCs had a higher apoptosis ratio than control cells (Figure 3A). The expression of caspase-3 and PARP was examined to identify the molecular mechanisms that underlie apoptosis induced in EL4 lymphoma cells co-cultured with hAT-MSCs. Caspases are a family of cysteine proteases that play essential roles in apoptosis, necrosis and inflammation (30). Caspase-3 is an effector caspase that cleaves other protein substrates within the cell to trigger the apoptotic process (30, 31). In this report, procaspase-3 levels decreased in EL4 cells co-cultured with hAT-MSCs by day 3 (Figure 3B). This experiment presupposed that up-regulated active caspase-3 lead to a down-regulation of procaspase-3 by day 3. PARP is a family of proteins involved in a number of cellular processes involving DNA repair and programmed cell death (32, 33). Upon DNA cleavage by enzymes involved in cell death (such as caspases), PARP depletes cellular ATP in an attempt to repair damaged DNA. ATP depletion in cells leads to lysis and cell death (32, 34). PARP can also directly induce apoptosis; this mechanism appears to be caspase-independent. In the present study, cleaved PARP was up-regulated in EL4 cells co-cultured with hAT-MSCs by day 3 (Figure 3B). These findings indicate that hAT-MSCs can trigger caspase-3 activation and thus PARP cleavage in EL4 lymphoma cells, ultimately leading to apoptotic death. PARP cleavage induced by hAT-MSCs may have been responsible for the observed tumor growth inhibition.

The T-cell lymphoma model was generated by injection of the mouse T-cell lymphoma cell line EL4 into the flanks of mice. When hAT-MSCs were administered circumtumorally in tumor-bearing nude mice, tumor growth was inhibited (Figure 4). Moreover, the enhanced life span of mice treated with hAT-MSCs was paralleled by a tendency for decreased tumor growth compared to the control group, but the observed differences were not significant (Figure 5). A similar study has reported that the treatment of a human lymphoma xenograft model with bone marrow mesenchymal stem cells (BM-MSCs) significantly reduced tumor volume (26). Confocal fluorescence images (Figure 6) indicated that hAT-MSCs migrated specifically to tumor sites; this indicates that T lymphoma cells may contain chemoattractant factors that enhance hAT-MSCs migration, although the precise molecular mechanisms by which the latter migrate to target tumor sites are not yet fully-understood. It has been reported that stromal cell-derived factor-1 (SDF-1)/CXCR4, stem cell factor (SCF)/c-Kit and/or vascular endothelial growth factor (VEGF)/VEGFR receptor (VEGFR) 1 and VEGFR 2, may play key roles in tumor-tropic effects (15). In addition to the intrinsic anti-tumor properties of MSCs, the presence of MSCs in tumor niches allows any secreted anti-tumor molecules to act locally and efficiently (35).
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References


