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Human mesenchymal stem cells (hMSCs) target osteosarcoma and promote its growth and pulmonary metastasis

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ABSTRACT

In an effort to study the interaction between MSCs and osteosarcoma, we established an animal model of primary osteosarcoma in nude mice using Saos-2 cells. hMSCs, labeled with adv-GFP, were injected through the caudal vein. We observed that exogenous hMSCs targeted the osteosarcoma site and promoted its growth and pulmonary metastasis *in vivo*. To elucidate the underlying mechanisms, we employed transwell, neutralization antibody and MTT assays *in vitro*. hMSCs migrated toward the conditioned medium from Saos-2 cells, and SDF-1 was involved in this migration. Likewise, Saos-2 cells migrated toward the conditioned medium from hMSCs and CCL5 played an important role in this migration. Furthermore, proliferation of Saos-2 cells was enhanced by the conditioned medium from hMSCs and CCL5 was at least partly responsible for this enhancement.

1. Introduction

Osteosarcoma (OS), which is sometimes referred to as osteogenic sarcoma, is the second most common primary malignant bone tumor, exceeded in frequency only by multiple myeloma. OS is characterized by high local aggressiveness with a tendency to metastasize to the lung and bone, and it accounts for approximately 15% of all primary bone tumours confirmed at biopsy [1]. Poor prognosis occurs in most patients due to lack of specific clinical symptoms in early stages of the disease. More importantly, more than half of patients treated by surgery alone develop metastases within six months, and more than 80% develop recurrent disease within two years of diagnosis without adjuvant chemotherapy [2]. Approximately 95% of patients who die of metastatic disease have metastasis in the lung indicated by autopsy [3]. Although integrated multimodal therapies have achieved great improvements in overall survival, the mechanisms underlying the development, progression, and metastasis of OS remain elusive. Thus, currently these areas are of intense research interest.

The non-hematopoietic mesenchymal stem cells (MSCs) in bone marrow were discovered by Friedenstein [4], who described clonal, plastic adherent cells from bone marrow capable of differentiating into osteoblasts, adipocytes and chondrocytes. In addition, these cells are stromal cells, which are structural components of bone marrow that support *ex vivo* culture of hematopoesis by providing extracellular matrix components, cytokines and growth factors [5–8]. Therefore, MSCs have considerable therapeutic potential in several disease processes, including cardiovascular disease [9], as well as in the treatment of human malignancies [10–14]. Recently, the relationship between MSCs and tumours has been highlighted by some interesting results. Hung et al. [15] demonstrated that MSCs could target microscopic tumours, subsequently proliferate and differentiate and then contribute to formation of a significant portion of tumor stroma in human colon cancer. Khakoo et al. [16] provided evidence that hMSCs exerted potent antitumorigenic

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effects in a model of Kaposi's sarcoma. The results from an elegant study by Karnoub et al. [17] showed that mesenchymal stem cells within tumor stroma promoted breast cancer metastasis. These observations indicate the complexity of the relationship between MSCs and tumours. And so far, there is little data on the relationship between MSCs and OS.

Chemokines are small soluble molecules that are best known for their potent abilities to induce cellular migration (for example, leukocytes in inflammation). Many types of cancer cells express chemokines and chemokine receptors [18]. In addition to inducing inflammatory cell infiltration into the tumor, local chemokines may regulate tumor cell migration or metastasis [19,20]. The CXC chemokine stromal cell-derived factor 1 (SDF-1 or CXCL12) [21] is expressed on the surface of vascular endothelial cells and is secreted by stromal cells from a variety of tissues such as bone marrow, lung and liver [22]. Its chemotactic effect is mediated by interaction with the chemokine receptor 4 (CXCR4 or CD184). It has been shown that endothelial cells expressing the CXCR4 receptor are strongly chemoattracted by SDF-1 [23]. Likewise, recent studies have shown that local production of the CC chemokine CCL5 (RANTES), a potent chemotactic factor for inflammatory cells, is important in the progression of breast cancer. A chemokine receptor antagonist of the CCL5 receptors CCR5 and CCR1 was recently shown to inhibit experimental breast tumor growth, further implicating CCL5 as an important molecule in breast cancer [24].

In order to study the interaction between MSCs and OS, we established an animal model of primary osteosarcoma by injecting Saos-2 cells into the proximal tibia of nude mice. After tumor formation, hMSCs labeled with green fluorescent protein (GFP) were injected through the caudal vein. Then, we monitored migration of exogenous hMSCs to the OS site and tumor development and progression, including growth and pulmonary metastasis in response to exogenous hMSCs. Finally, we employed *in vitro* experiments including transwell, MTT and neutralization antibody assays to investigate the possible mechanisms responsible for the phenomena observed *in vivo*, focusing on SDF-1 and CCL5.

2. Materials and methods

2.1. Cell cultures and adenovirus infection

The hMSCs were isolated and expanded using a modification of methods as described previously [25,26]. The donor was healthy without metabolic disease, inherited illnesses, or other diseases that may affect the current study. Cells were grown in complete Alpha Minimum Essential Medium (α-MEM; GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Tauranga, New Zealand) and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml; Hyclone, Logan, UT, USA) in 37 °C humidified atmosphere with 5% CO_2. Passage 3 hMSCs were incubated with adenovirus-GFP with 150 multiples of infection (MOI = 150) at 37 °C in 5% CO_2 overnight. Infected cells were continuously cultured in complete α-MEM with 10% FBS after removal of infection medium.

Human Saos-2 cells were purchased from Chinese Academy of Sciences (Shanghai) and grown in α-MEM (GIBCO, Grand Island, NY, USA) supplemented with 10% FBS (Hyclone, Tauranga, New Zealand) and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml; Hyclone, Logan, UT, USA) in 37 °C humidified atmosphere with 5% CO_2.

2.2. Tumor xenografts in nude mice

Study protocols involving mice were approved by the Animal Ethics Committee of Shanghai Jiaotong University School of Medicine. 33 Bacb/c male nude mice (four weeks of age) were purchased from Chinese Academy of Sciences (Shanghai), which were divided into three groups: hMSCs group ($n = 10$), OS group ($n = 11$) and OS + hMSCs ($n = 12$). Saos-2 cells, resuspended in serum-free α-MEM to a final concentration of 2×108 cells/ml, were injected into the right proximal tibia of nude mice at a dose of 1×107 per injection site [27]. Four weeks later, hMSCs of 2×107 cells/ml labeled with adenovirus-GFP were injected through the caudal vein of nude mice at a dose of 1×106 per mouse. After eight weeks of hMSCs injection, mice were sacrificed by excess pentobarbital. Lung, *in situ* tumor and blood for ALP quantification assay [26] were harvested for further analysis.

After injection of exogenous hMSCs through the caudal vein, the volume of OS and weight of each mouse were measured at one-week intervals until sacrifice of animal. The volume of tumor was calculated by using the following equation as reported previously [27]: volume = $0.2618 \times L \times W \times (L + \mathrm{W})$. W was an average of the distance at the proximal tibia at the level of the knee joint in the anterior–posterior and medial–lateral planes. L was the distance from the most distal extent of the distal tumor margin to the proximal tumor margin.

2.3. Immunohistochemistry

Briefly, frozen sections were treated with 3% hydrogen peroxide in methanol for 10 min to inactivate endogenous peroxidases, then treated with primary antibody against GFP (Invitrogen, A11122, USA), SDF-1 (Biovision 5388-100, USA) or CCL5 (Peprotech Inc, Cat#: 500-P36, USA) overnight at 4 °C. After rinsing with PBS, sections were treated for 20 min with pre-diluted biotin-conjugated broad-spectrum IgG secondary antibody (Zymed Laboratories, South San Francisco, CA, USA), and then visualized using streptavidin conjugated horseradish peroxidase provided with the Zymed Streptavidin–Biotin System Histostain-SP Kit following instructions specified by the manufacturer.

2.4. ELISA and neutralization antibody assay

To determine SDF-1 or CCL5 secretion in the conditioned medium from Saos-2 cells or hMSCs, cells were plated in medium containing 10% FBS. The medium was changed at day 3 and 5. After the cells reached confluence,

the supernatants were collected according to the manufacturer's instructions. The media were analyzed by SDF-1 ELISA kit (Raybio, ELH-SDF alpha-001, USA) and CCL5 ELISA kit (Invitrogen, KHC1032, USA). To impede the activity of SDF-1 or CCL5, SDF-1 neutralization antibody (Peprotech Inc, 500-P87A, USA) or CCL5 neutralization antibody (Peprotech Inc, 500-P36, USA) was added to the conditioned medium from Saos-2 cells or hMSCs.

2.5. Transwell assay

The migration potential was evaluated using transwell chambers (Corning, USA). The upper and lower cultures were separated by 8-μm pore size polyvinylpyrrolidone-free polycarbonate filters. Briefly, the lower compartment of the chamber was loaded with aliquots of the conditioned medium with or without neutralization antibody. 2×10^4 cells in 200 μl α-MEM/10% FBS were put in the upper compartment. The chamber was cultured in a humidified 37 °C with 5% CO_2 incubator for 16 h. Then cells migrating from the upper compartment were fixed with ethanol and stained with hematoxylin. Each experiment was done in triplicate. Cells were counted in five fields in each well by light microscopy. And mean cell number of five fields in each well was used as statistic.

2.6. MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was done according to the manufacturer's instructions (Sigma–Aldrich). Briefly, 1×103 Saos-2 cells were seeded in a 96-well plate in 150 μl conditioned medium from hMSCs with or without neutralization antibody against CCL5. At each indicated time point, MTT solution was added to each well and plates were incubated for 3–4 h; subsequently, DMSO (Sigma–Aldrich) was added to the wells for 5 min. The plates were then read at 570 nm using an automated plate reader (Perkin–Elmer).

2.7. Statistical analysis

The results were expressed as means ± S.D. Data were analyzed using ANOVA and chi square tests. *p*-values less than 0.05 were considered significant.

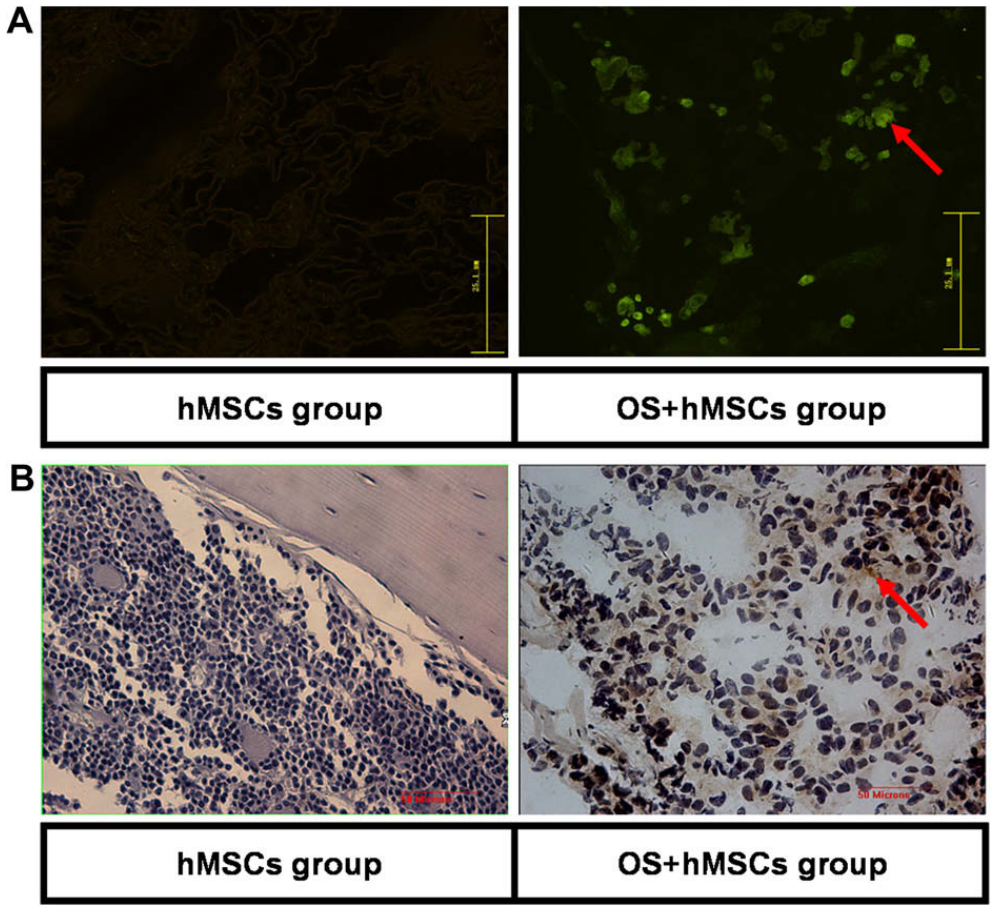

Fig. 1. In vivo migration of exogenous hMSCs to OS site. An animal model of primary OS was established in the proximal tibia of nude mice using Saos-2 cells. After tumor formation (~4 weeks), hMSCs labeled with adv-GFP were injected through caudal vein. 8 weeks later, the tumor was recovered and GFP expression was cross-validated by both fluorescence microscope (A) and immunohistochemistry assay using antibody against GFP (B). The sections of proximal tibia of nude mice only receiving hMSCs injection through caudal vein without OS tumor were used as control to compare OS + hMSCs sections. Red arrows indicate GFP presence. (For interpretation of color mentioned in this figure legend the reader is referred to the web version of the article.)

3. Results

3.1. In vivo migration of hMSCs to OS site

In order to study the relationship between MSCs and OS, we established an animal model of primary osteosarcoma in proximal tibia of nude mice using Saos-2, a cell line that is widely used in tumor research. After tumor formation (~4 weeks), hMSCs labeled with GFP were injected through the caudal vein. 8 weeks later, the tumor was recovered and its frozen sections were inspected and photographed under fluorescence microscopy to monitor GFP expression. The result showed that obvious green fluorescence was observed within the tumor site (Fig. 1A), which was supported by the result from the immunohistochemistry experiment in which antibody against GFP was hybridized to the same section (Fig. 1B). And green fluorescence was not observed in proximal tibia of nude mice only receiving hMSCs injection through the caudal vein without OS tumor (Fig. 1). Taken together, these observations implied that exogenous hMSCs had the ability to migrate to the OS site *in vivo*.

3.2. Possible mechanisms underlying the migration of exogenous hMSCs to the OS site

We asked what mechanisms were responsible for our observation that exogenous hMSCs injected through the caudal vein could migrate toward the OS site in nude mice *in vivo*. To answer this question, we focused on SDF-1, which is involved in tumor progression of breast, prostate, and pancreatic cancer as well as in rhabdomyosarcoma and lymphoma [20,28–30]. In the present study, *in vivo* expression of SDF-1 at the tumor site of nude mice was shown by immunohistochemistry using antibody

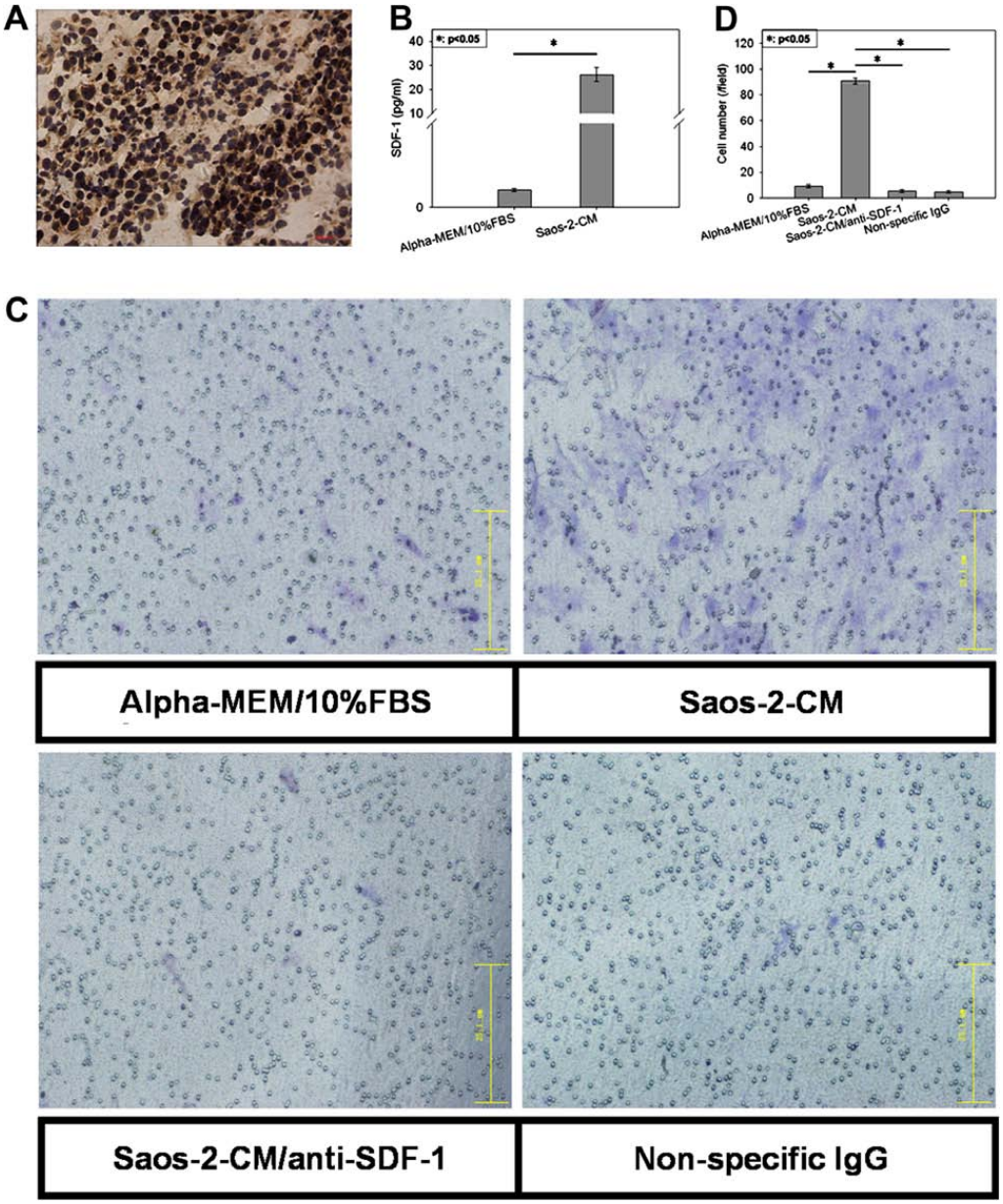

Fig. 2. The involvement of SDF-1 in the migration of hMSCs towards the conditioned medium from Saos-2 cells. Immunohistochemistry assay using antibody against SDF-1 to hybridize the section of OS was preformed to declare SDF-1 expression *in vivo* (A). And *in vitro* SDF-1 expression of the conditioned medium from hMSCs was measured through ELISA (B). Transwell assay using hMSCs cells and the conditioned medium from Saos-2 cells (Saos-2-CM) in combination with neutralization antibody against SDF-1 was employed (C and D). Each experiment was done in triplicate. Cells were counted in five fields in each well by light microscopy. And mean cell number of five fields in each well was used as statistic. The results were expressed as mean ± S.D. $^{*}p < 0.01$.

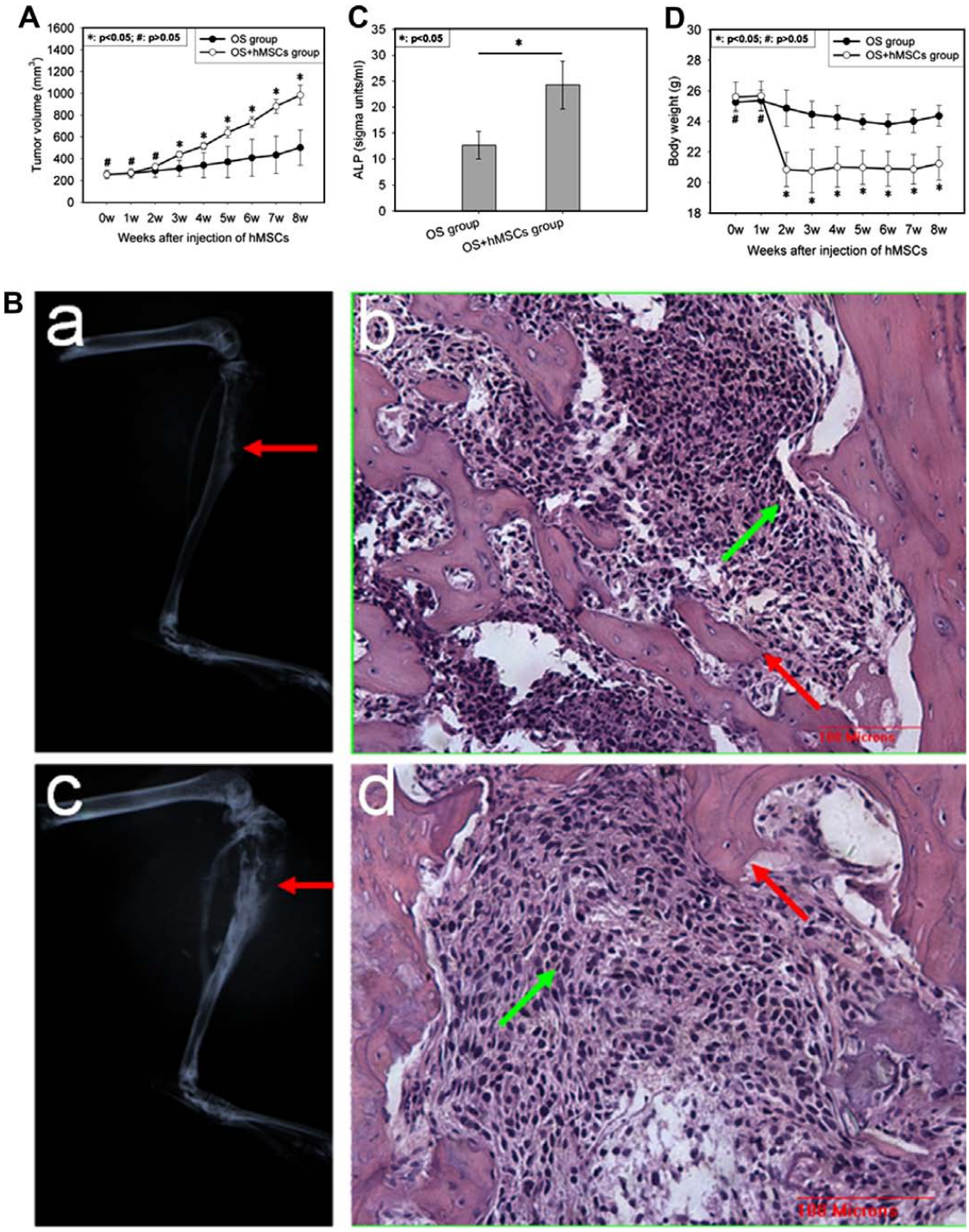

Fig. 3. Development and progression of OS *in vivo* in response to exogenous hMSCs. Since injection of exogenous hMSCs through caudal vein, the volume of OS (A) and the body weight of nude mice (D) were measured at 1-week interval until sacrifice of animal. The results were expressed as mean ± S.D. $^{*}p < 0.05$; $^{\#}p > 0.05$, OS + hMSCs vs. OS. After sacrifice of animal, X-ray photograph and histochemistry assay of tumor (B) without (a and b) or with (c and d) exogenous hMSCs were performed. And the blood from nude mice in both OS and OS + hMSCs groups was recovered and the level of alkaline phosphatase (ALP) in it was measured (C). The results were expressed as mean ± S.D. $^{*}p < 0.05$. Red arrows indicate osteolytic lesions. Green arrows indicate OS cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

against SDF-1 (Fig. 2A). And *in vitro* expression of SDF-1 in the conditioned medium from Saos-2 cells was measured by ELISA (Fig. 2B). To assess the role of SDF-1 in hMSCs migration to OS site, we employed transwell assay using hMSCs and the conditioned medium from Saos-2 cells in combination with neutralization antibody against SDF-1 *in vitro*. The results from transwell assay showed that the migration of hMSCs was promoted by the conditioned medium from Saos-2 cells. Addition of neutralization antibody against SDF-1 to the conditioned medium of

Table 1
Pulmonary metastasis rate with or without exogenous hMSCs.

Group	Total	Pulmonary metastasis	No metastasis	Metastasis rate (%)	*p* value
OS	11	3	8	27.27	$p < 0.01$
OS + hMSCs	12	11	1	91.67	

Saos-2 cells impaired this migration of hMSCs (Fig. 2C and 2D). These results suggested that the migration of hMSCs toward Saos-2 cells is dependent (at least in part) on SDF-1 in the conditioned medium from Saos-2 cells and offered a possible explanation for hMSCs migration towards OS site *in vivo*, that is, Saos-2 cells in proximal tibia of nude mice secreted SDF-1 to recruit hMSCs towards OS site.

3.3. Development and progression of OS in vivo in response to exogenous hMSCs

In addition to observing that exogenous hMSCs migrated to OS site *in vivo*, we monitored the development and progression of OS in response to exogenous hMSCs, including OS growth and pulmonary metastasis.

After injection of exogenous hMSCs through the caudal vein, the volume of OS was measured at one-week intervals until sacrifice of the animal. There was no change in tumor volume with or without exogenous hMSCs injection during the first two weeks. However, enhanced tumor volume was observed from week 3 to 8 after injection of hMSCs (Fig. 3A). This observation was supported by both X-ray photography and histochemistry assays of tumours at week 8 after injection of hMSCs, which indicated more severe osteolytic lesions and periosteal reactions in the proximal tibia and increased tumor size in response to exogenous hMSCs relative to OS group (Fig. 3B). Furthermore, the level of ALP in blood serum was measured at week 8 after injection of hMSCs to determine the progression of OS. Statistically significant enhancement of ALP level was observed in response to exogenous hMSCs compared to OS

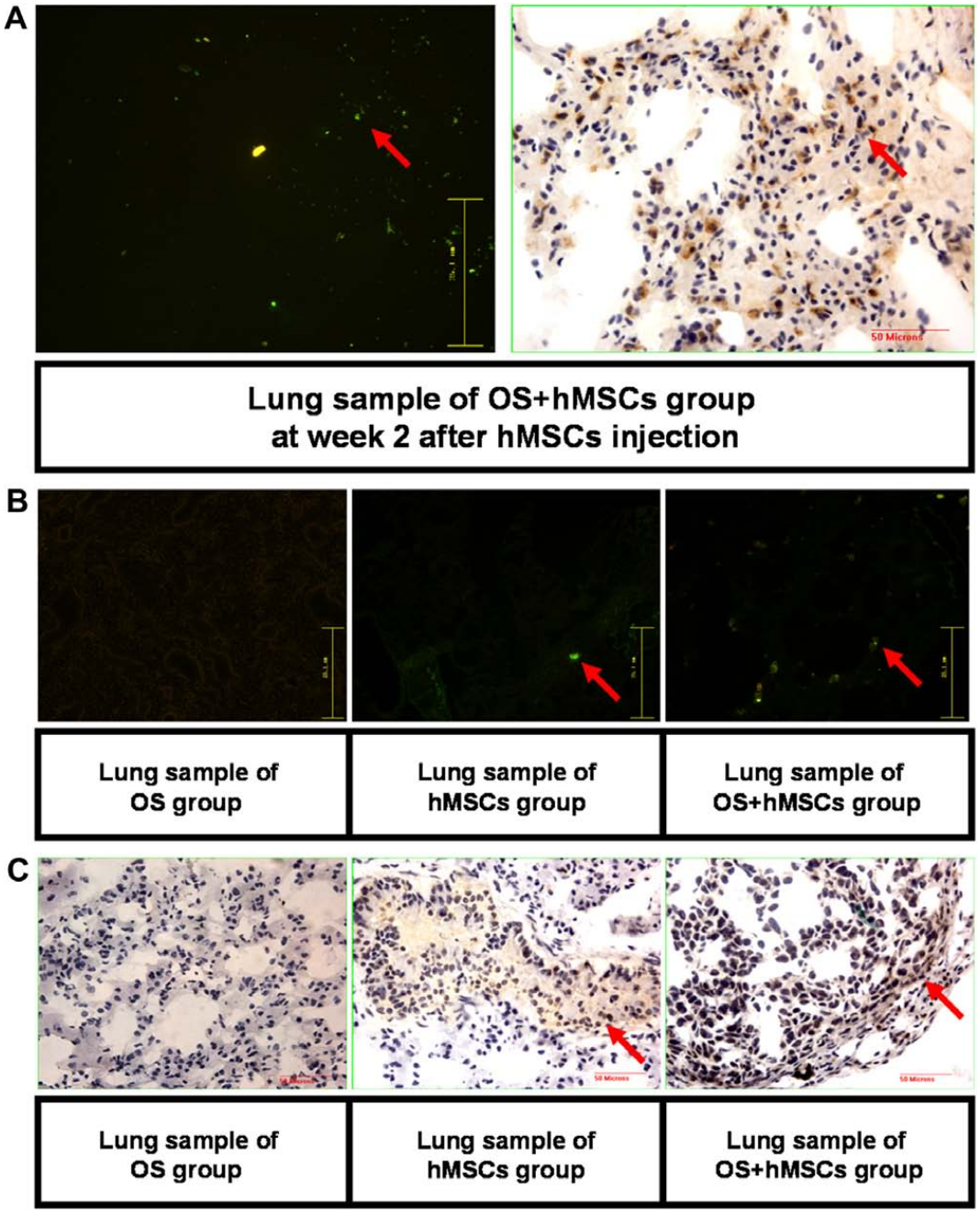

Fig. 4. Pulmonary metastasis of OS in response to exogenous hMSCs. An animal model of primary OS was established in the proximal tibia of nude mice using Saos-2 cells. After tumor formation (~4 weeks), hMSCs labeled with adv-GFP were injected through caudal vein. 2 weeks later, lung sample of nude mice was recovered and sections were inspected by both fluorescence microscope and immunohistochemistry assay using antibody against GFP (A). At week 8 after hMSCs injection, lung samples of OS, hMSCs and OS + hMSCs groups were recovered and sections were inspected by both fluorescence microscope (B) and immunohistochemistry assay using antibody against GFP (C). Red arrows indicate GFP expression. (For interpretation of color mentioned in this figure legend the reader is referred to the web version of the article.)

group (Fig. 3C). In addition, body weight of each animal was monitored at one-week intervals after hMSCs injection. There was a more remarkable decrease in body weight in OS + hMSCs group than OS group (Fig. 3D).

Interesting data were obtained from measurement of the pulmonary metastasis rate of OS with or without exogenous hMSCs. There was a remarkably higher rate of pulmonary metastasis in OS + hMSCs compared to OS group (Table 1). Considering the accumulation of a large portion of exogenous hMSCs injected through the caudal vein in the lung at week 2 after injection which preceded pulmonary metastasis of OS tumor (Fig. 4A), we postulated that Saos-2 cells in the proximal tibia were driven to undergo pulmonary metastasis by components that were secreted by exogenous hMSCs accumulating in the lung. And pulmonary metastasis of tumor was determined by histochemistry assay using sections of lung sample (Figs. 4B and 4C).

Taken together, these observations implied that the development and progression of OS, including tumor growth and pulmonary metastasis, were promoted in response to exogenous hMSCs.

3.4. The possible mechanisms by which OS growth was promoted in response to exogenous hMSCs

After observing the enhancement of OS growth in response to exogenous hMSCs *in vivo*, we set out to study the mechanisms responsible for this phenomenon. In fact, hMSCs migrating from caudal vein to the tumor site and merging with tumor stroma, observed in the aforementioned section, contributed the enhancement of OS growth. However, there was another possibility that component(s) secreted by hMSCs promoted OS growth. We focused our attention on CCL5 secreted by hMSCs, whose in vitro expression was measured by ELISA (Fig. 6B). MTT assay was performed using Saos-2 cells and the conditioned medium from hMSCs in combination with neutralization antibody against CCL5 to assess its role in the proliferation of Saos-2 cells in response to the conditioned medium of hMSCs. As Fig. 5 indicates, the proliferation of Saos-2 cells was enhanced by the conditioned medium from hMSCs, and neutralization antibody against CCL5 eliminated this enhancement, suggesting that CCL5 secreted by hMSCs was responsible (at least in part) for the enhancement of proliferation of Saos-2 cells by the conditioned medium from hMSCs.

3.5. The possible mechanisms responsible for pulmonary metastasis of OS in response to hMSCs

Having observed that exogenous hMSCs enhanced the rate of pulmonary metastasis of OS, we sought to elucidate the underlying mechanisms responsible for it. Considering the accumulation of a large portion of exogenous hMSCs injected through the caudal vein in the lung which preceded pulmonary metastasis of OS tumor (Fig. 4A), we postulated that Saos-2 cells in the proximal tibia were driven to undergo pulmonary metastasis by components that were secreted by exogenous hMSCs accumulating in the lung. To test this hypothesis, we employed transwell assay using Saos-2 cells and the conditioned medium from hMSCs in combination with neutralization antibody against CCL5. *In vivo* expression of CCL5 of hMSCs injected through caudal vein and accumulating in lung of nude mice was demonstrated in immunohistochemistry assay using antibodies against CCL5 (Fig. 6A). And *in vitro* expression of CCL5 in the conditioned medium from hMSCs was uncovered by ELISA (Fig. 6B). The results from the transwell assay showed that the conditioned medium from hMSCs promoted the migration of Saos-2 cells. Addition of neutralization antibody against CCL5 to the conditioned medium of hMSCs impeded this migration of Saos-2 cells (Fig. 6C and 6D). These results suggested that CCL5 secreted by hMSCs played an important role in the migration of Saos-2 cells toward the conditioned medium from hMSCs.

4. Discussion

The aim of the current report was to study the interaction between MSCs and OS. To achieve it, we established an animal model of primary OS in nude mice using Saos-2 cells. After tumor formation hMSCs labeled with GFP were injected through the caudal vein. The presence of GFP was observed within the tumor site, suggesting that exogenous

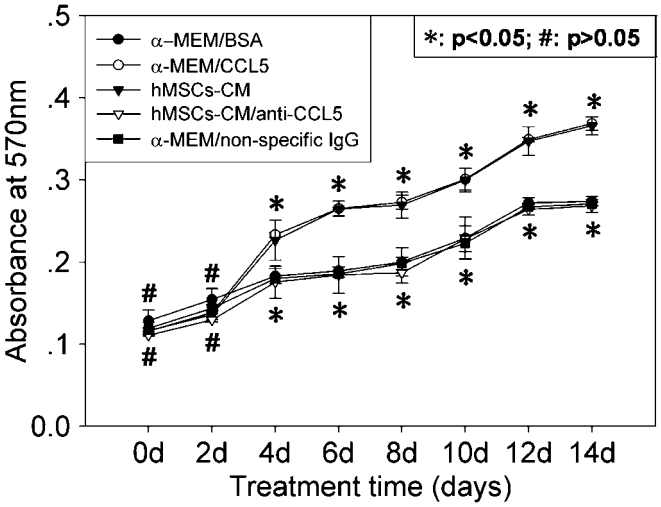

Fig. 5. The involvement of CCL5 in the enhancement of proliferation of Saos-2 cells stimulated by the condition medium from hMSCs. MTT assay using Saos-2 cells in combination with α-MEM/BSA, α-MEM/CCL5, the conditioned medium from hMSCs (hMSCs-CM), hMSCs-CM/anti-CCL5 or α-MEM/non-specific IgG was performed and at indicated time point, the absorbance of cell lysates at 570 nm was read. The results were expressed as mean ± S.D. $^{*}p < 0.05$; $^{\#}p > 0.05$.

hMSCs could migrate to OS. Enhanced tumor growth was observed in response to the injection of hMSCs. Furthermore, there was a higher pulmonary metastasis rate in OS + hMSCs group compared to OS group. We employed transwell, neutralization antibody and MTT assays *in vitro*. hMSCs migrated toward the conditioned medium from Saos-2 cells, and SDF-1 secreted by Saos-2 cells was involved in this migration of hMSCs. Likewise, Saos-2 cells migrated toward the conditioned medium from hMSCs, and CCL5 secreted by hMSCs played an important role in this migration. Finally, the proliferation of Saos-2 cells was enhanced by the conditioned medium from hMSCs, and involved CCL5.

Using fluorescence microscopy and an immunohistochemistry assay with antibody against GFP, we determined that exogenous hMSCs injected through the caudal vein could migrate towards the OS site located in the proximal tibia. Cancer cells are known to produce various chemokines that selectively attract and activate different cell types. Chemokines are a superfamily of cytokine-like proteins that are grouped into CXC and CC chemokines. Through their interaction with G-protein-coupled receptor, these secreted proteins induce cytoskeleton rearrangement, firm adhesion to endothelial cells and directional migration [21,31]. Recently, the interaction between the chemokine receptor CXCR4 and its ligand, stromal cell-derived factor 1 (SDF-1), has been found to play important roles in tumorigenicity, proliferation, metastasis, and angiogenesis in many cancers such as lung cancer, breast cancer, melanoma, glioblastoma, pancreatic cancer and cholangiocarcinoma [32–37]. In the current study, we provided evidence that the migration of hMSCs was promoted in response to the conditioned medium from Saos-2 cells. More importantly, we found that SDF-1, which was secreted by Saos-2 cells, played an important role in this migration of hMSCs.

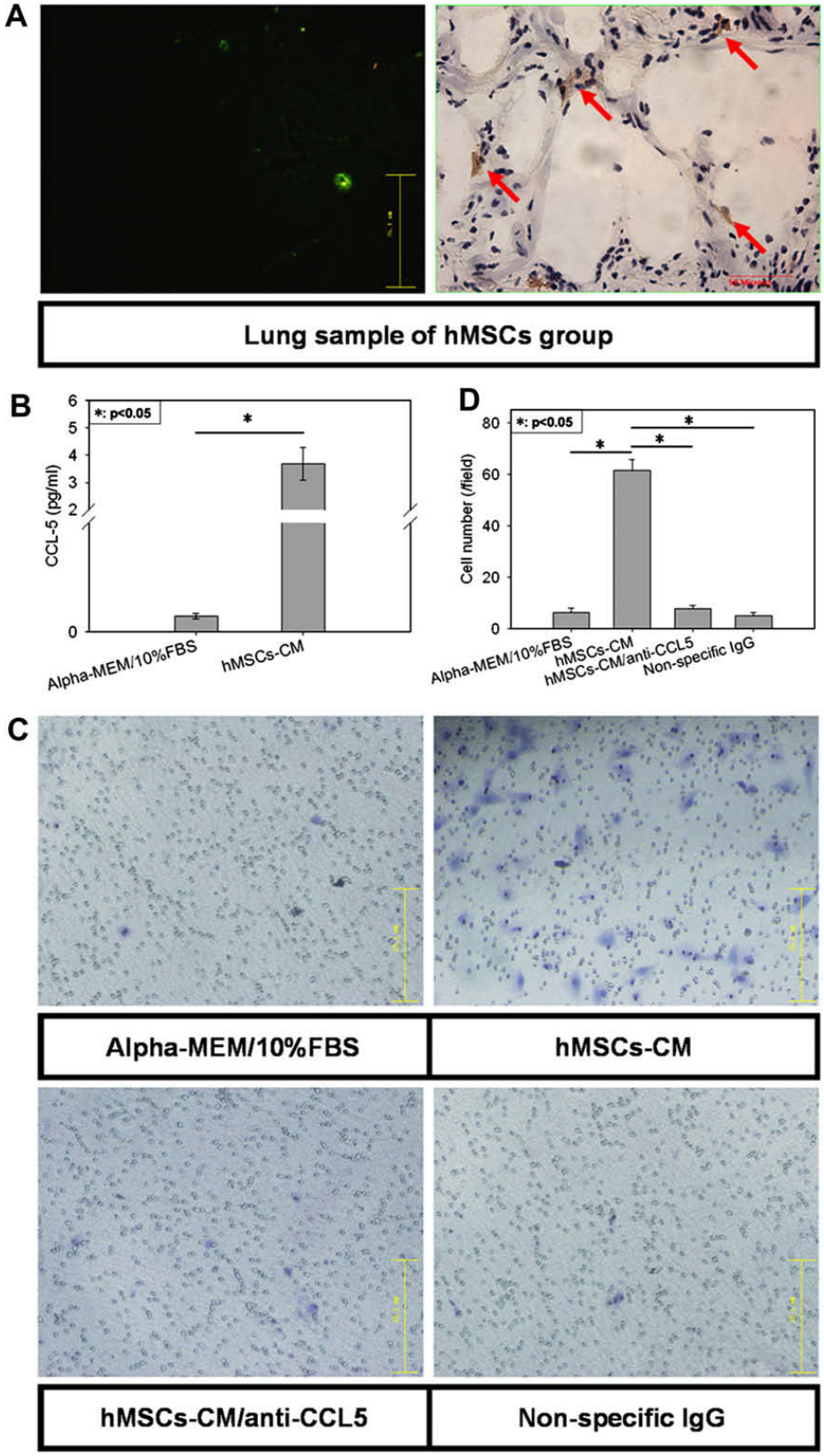

Fig. 6. The role of CCL5 in the migration of Saos-2 cells towards the conditioned medium from hMSCs. At week 2 after hMSCs injection through caudal vein, lung of nude mice was recovered and fluorescence microscope and immunohistochemistry assay using antibody against CCL5 were preformed to exhibit GFP and CCL5 expression *in vivo* (A). And *in vitro* CCL5 expression of the conditioned medium from hMSCs was measured through ELISA (B). Transwell assay using Saos-2 cells and the conditioned medium from hMSCs (hMSCs-CM) in combination with neutralization antibody against CCL5 was employed (C and D). Each experiment was done in triplicate. Cells were counted in five fields in each well by light microscopy. And mean cell number of five fields in each well was used as statistic. The results were expressed as mean ± S.D. $^{*}p < 0.01$.

Tumor-targeting properties of stem cells have been reported previously in a glioblastoma model [38] and in studies of tumor angiogenesis by hematopoietic stem cells [39]. Stem cells have been used as vehicles for gene therapy of human colon cancer xenografts [14]. This phenomenon forms the basis for the paradigm of the "Trojan Horse" approach, in which adult stem cells are used as shuttle vectors for delivery of gene therapies into growing tumours. If proven clinically effective, such an approach may provide the means for treatment of metastatic tumours in the future.

However, based on these observations alone, it would be irresponsible and risky to assert that MSCs is an eligible tool for treatment of tumours. So far we know little about the interaction between MSCs and tumours. And the results of our report provided evidences that an intricate interaction existed between hMSCs and OS.

On one hand, enhanced OS growth was observed in response to the injection of hMSCs. We offer two possible explanations for this result. Firstly, exogenous hMSCs enhanced OS growth by their migration from the caudal vein to the tumor site and mergence with tumor stroma, which was observed in the current report. It is known that MSCs could produce various growth factors that stimulate angiogenesis [40]. In response to these factors, endothelial precursors, including bone marrow-derived endothelial progenitors [41] and hematopoietic stem cells [39], may be recruited into the tumor neovascularization process. So, it is possible that factors secreted by hMSCs which have merged with OS might induce angiogenesis of OS, which led to enhanced blood supply and growth of the tumor. Secondly, the other explanation is that factors secreted by exogenous hMSCs directly stimulate the growth of OS. In the present report, we provided evidences that the proliferation of Saos-2 cells was enhanced by the conditional medium from hMSCs *in vitro* and that CCL5 secreted by hMSCs was responsible for this phenomenon at least in part. CCL5, originally identified as a product of activated T cells, is capable of recruiting T cells to inflammatory sites [42,43]. However, there is little data on the effect of CCL5 on proliferation of cancer cells. In agreement with the present report, a study by Adler et al. [44] demonstrated that tumours expressing low levels of CCL5 exhibited decreased growth rates.

On the other hand, interesting results were obtained involving the pulmonary metastasis rate in response to exogenous hMSCs in this report. A remarkably higher rate of OS pulmonary metastasis was observed in response to exogenous hMSCs. The possible reason is that factors secreted by exogenous hMSCs induced Saos-2 cells to undergo pulmonary metastasis *in vivo*. Indeed, remarkable accumulation of exogenous hMSCs in the lung was observed after a period of injection through the caudal vein. Consistent with our result, a study by Kyriakou et al. [45] demonstrated that exogenous hMSCs administered by vein were readily detected in bone marrow, spleen, liver and lungs at 20–24 h after infusion. In an effort to identify factors secreted by hMSCs that have the ability to chemoattract Saos-2 cells, we focused on CCL5. The results from *in vitro* assays showed that neutralization antibody against CCL5 impaired the migration of Saos-2 cells to the conditioned medium from hMSCs. The other possible reason is that exogenous hMSCs that migrated to the tumor site and merged with tumor stroma stimulated pulmonary metastasis of OS. This hypothesis is supported by the study from Karnoub et al. [17] that demonstrated that MSCs within tumor stromal cells promote breast cancer metastasis.

Taken together, we made a conclusion that exogenous hMSCs targeted the OS site and promoted its growth and pulmonary metastasis *in vivo*. We provided evidences that SDF-1 and CCL5 played essential roles in the interaction between hMSCs and OS.

Conflicts of interest statement

None declared.

Acknowledgments

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