Mesenchymal stem cells promote tumor engraftment and metastatic colonization in rat osteosarcoma model

SHINJI TSUKAMOTO[1], KANYA HONOKI[1], HIROMASA FUJII[1], YASUAKI TOHMA[1], AKIRA KIDO[1], TOSHIO MORI[2], TOSHIFUMI TSUJIUCHI[3] and YASUHITO TANAKA[1]

[1]Department of Orthopedic Surgery, Nara Medical University, 840 Shijo-cho, Kashihara; [2]RI Center, Nara Medical University, 840 Shijo-cho, Kashihara 634-8521; [3]Department of Life Sciences, Faculty of Science & Technology, Kinki University, Kowakae 3-4-1, Higashiosaka 577-8502, Japan

Received July 4, 2011; Accepted August 18, 2011

DOI: 10.3892/ijo.2011.1220

Abstract. Although mesenchymal stem cells (MSCs) are considered to be the cells of origin for most sarcomas, the role of MSCs as a source of tumor stroma is not fully understood in this tumor type. The current study investigated whether MSCs affect the tumor growth and metastatic ability in rat osteosarcoma model. Results from subcutaneous co-implantation of rat osteosarcoma COS1NR cells, established in our laboratory, with rat MSCs isolated from femur bone marrow showed that the incidence of tumor formation and tumor growth rate was higher until 5 weeks compared to COS1NR cell inoculation alone. However, no difference was observed in tumor growth afterwards and in the number of metastatic nodules at 9 weeks (0.75 vs. 1.2). Intravenous MSC injection at weeks 3 and 5 after subcutaneous inoculation of COS1NR cells significantly increased the number of lung nodules in the group with MSC injection compared to the group without MSC injection (17.33 vs. 2.0), while no difference was observed in subcutaneous tumor growth between those groups. Pathway analysis from gene expression profile identified that genes involved in focal adhesion, cytokine-cytokine receptor and extracellular matrix-receptor pathways such as CAMs (ICAM and VCAM)-integrins were highly expressed in MSCs, possibly participating in the tumor progression of osteosarcoma. These results suggest that MSCs could provide a source of microenvironments for osteosarcoma cells, and might enhance the ability of settlement and colonization which lead to early onset of growth and metastasis, possibly through their activated pathways interaction.

Correspondence to: Dr Kanya Honoki, Department of Orthopedic Surgery, Nara Medical University, 840 Shijo-cho, Kashihara 634-8521, Japan
E-mail: kahonoki@naramed-u.ac.jp

Key words: mesenchymal stromal cell, osteosarcoma, cancer stem cell niche, metastasis, rat

Introduction

Historic landmark of tumor microenvironment research was proposed as the 'seed and soil' hypothesis by Stephen Paget 120 years ago (1), and the importance of the microenvironment and stroma in the evolution and progression of solid tumors has drawn renewed interest in recent years. A complex structure of mixed cell types and tissues with endothelial cells, immune cells, stromal cells and extracellular matrix is essential for the growth and progression of solid tumors (2). Although all of these components in the tumor are integral in carcinogenesis and metastasis, the stroma is considered to be a 'co-conspirator' in the evolution and progression of the disease (3). Among the stromal elements, the progenitor cells of the stroma, the mesenchymal stem cells (MSCs), have been receiving focused attention lately.

Mesenchymal stem cells (MSCs) are mainly derived from bone marrow, non-hematopoietic precursor cells possessing differentiation potential to skeletal mesodermal lineages such as osteoblasts, chondrocytes and adipocytes as well as some extra-mesodermal cell types including neural, pancreatic and hepatocytic phenotypes with strong proliferative capacity (4-6), and are of increasing interest as the future therapeutic tool in regenerative medicine (7,8). MSCs also display immune suppressive properties which could be the potentially exploited for therapeutic treatment of auto-immune diseases and the reduction of graft-versus host disease (9,10).

Besides these beneficial effects of MSCs, they show a potential unwanted effect on tumor growth and progression. MSCs have been shown to have homing capacity to sites of inflammation and injury as well as homing to bone marrow (11,12), and they can migrate across the endothelium in a similar manner to leukocyte trafficking (13). Several studies have shown that MSCs can be found preferentially migrating to tumors and even to sites of metastasis (14,15). The interaction between MSCs and tumor cells are not limited to homing, but also promote tumor progression. Several reports suggested MSCs could contribute to the growth and progression of some malignant tumors such as melanoma, adenocarcinoma, and breast cancer (16-18) through the modulation of immune system (9,16) and tumor microenvironment with angiogenic induction (19,20) as well as direct interaction with tumor cells. In this context, MSCs may target microscopic tumors, and lead them to proliferation and

differentiation with the tumor stroma formation (21). Various adhesion molecules and chemokines including integrins and CXCL12-CXCR4 axis are involved in MSCs migration and tumor progression; however, the detailed mechanism by which MSCs interact with tumor cells remains unknown.

Sarcomas are relatively rare, and many types tend to occur in childhood and adolescents with poor prognosis. Most of sarcomas, probably not all, are considered to be originated from mesenchymal stem/progenitor cells (22), and there have been several reports indicating MSCs could transform some type of sarcoma cells (23-25). However, there have been few reports regarding the MSCs as tumor stroma in sarcoma tissues (26).

In the current study, we investigated the interaction between MSCs and sarcoma cells whether MSCs might affect tumor growth and metastatic ability using rat osteosarcoma model by co-implantation and intravenous injection of MSCs. The results indicated that MSCs may promote the settlement, i.e. increased incidence of tumor formation and early growth of rat osteosarcoma cells in the process of tumor development including metastasis.

Materials and methods

Cell lines. Rat osteosarcoma cell line COS1NR was established from chemically-induced osteosarcoma in Fischer 344 rats by 4-hydroxy quinolone 1-oxide in our laboratory (27,28). Rat mesenchymal stem cells (MSCs) were isolated from rat femur bone marrow through the monolayer culture on a plastic dish (29). COS1NR cells are maintained in Dulbecco's minimum essential medium with 10% fetal bovine serum, and MSCs are maintained in Dulbecco's minimum essential medium with 15% fetal bovine serum in an atmosphere of 5% CO_2 at 37˚C.

In vivo tumor formation in syngeneic rats.
Experiment I: Cells ($5x10^6$) of COS1NR alone (Group 1), MSCs alone (Group 2) and both cells combined (Group 3) in 100 μl of phosphate buffer saline (PBS) were inoculated into subcutaneous tissue in the back of Fischer 344 rats. Each group consisted of 5 rats and evaluated for incidence of tumor formation; the growth rate of tumor formed every week and formation of lung metastatic nodules at 9 weeks. The size of tumors were calculated by the formula of volume = 0.2618xLxWx (L+W) (30). The number of lung metastatic nodules was counted macroscopically. Samples from subcutaneous tumors and lung metastatic lesions in each group were fixed in 3.7% formaldehyde neutral buffer solution, and then processed routinely for histology, stained with hematoxylin-eosin and examined under light microscopy.

Experiment II: Cells ($5x10^6$) of COS1NR were inoculated subcutaneously as described in Experiment I. Subsequently, the same number of MSCs in 100 μl of PBS were directly injected into the circulation through the tail vein twice at weeks 3 and 5 (Group 1). The growth rates of subcutaneous tumors and the lung metastatic nodule formation were evaluated as described above, and compared to the control group without MSCs injection (Group 2). Histological evaluation was also performed as described above. All animals were housed in our facilities and cared in accordance with the institutional laboratory animal care guidelines.

Cell migration assay. The migration potential was evaluated using Cytoselect™ 96-Well Cell Invasion Assay (Cell Biolabs, Inc., San Diego, USA), which contains polycarbonate membrane inserts (8 μm pore size) coated with a uniform layer of extracellular matrix solution. Invasive cells are able to degrade the matrix protein, and pass through the pores of the polycarbonate membrane. The experiment was performed according to the manufacturer's instructions. Briefly, $1x10^5$ COS1NR cells/well in 100 μl DMEM-10% FBS were seeded in the upper compartment, and the lower compartment of the chamber was loaded with the same number of MSCs with DMEM-10% FBS or conditioned medium from cultured MSCs and DMEM-10% FBS alone as a control. The chamber was cultured in 5% CO_2 incubator for 24 h. The invaded cells were dissociated from the membrane and subsequently detected with CyQuant GR Dye.

Gene expression profiling of COS1NR and MSCs. To investigate the difference of gene expression between osteosarcoma cells and MSCs, the gene expression profiling were performed by Agilent array analysis (Agilent Technologies, Böblingen, Germany). Total RNA was isolated from both COS1NR cells and MSCs, and underwent quality assessment by Agilent 2100 Bioanalyzer. Total RNA (500 ng) was processed to Agilent expression array analysis using Quick Amp Labeling Kit and Gene Expression Hybridization Kit. Data analysis was carried out using Agilent Feature Extraction software, analyzing pathways that possibly interact in osteosarcoma cells and MSCs.

Statistical analysis. Statistical analysis was performed with Student's t-test using STATA8 for tumor volume, lung metastatic nodules and cell migration ability.

Results

Earlier onset of syngeneic tumor development after co-implantation of osteosarcoma cells and MSCs. We investigated the effect of rat mesenchymal stem cells (MSCs) on the behavior of tumor cells after implantation in syngeneic Fischer 344 rats, comparing the growth of COS1NR rat osteosarcoma cells between co-implantation with and without MSCs. COS1NR cells co-implanted with MSCs developed tumors in 100% of the animals by week 4, compared to week 6 when implanted alone, indicating earlier onset of tumor growth occurred in the presence of MSCs (Table I). In parallel to incidence, tumor growth was assessed by the measure of the tumor volume and the lung metastatic nodule formation was evaluated at week 9. There were no significant statistical variation in tumor volume observed between the groups with and without co-implantation of MSCs after 6 weeks (Fig. 1a, 52643.2±15369 vs. 38885.7±29530.9 mm^3 at 9 week, p=0.38). There were no statistically significant differences observed in the number of lung metastatic nodules between the groups with and without co-implantation of MSCs (1.2±1.31 vs. 0.75±0.96, p=0.61), neither were differences observed in histological appearance between these groups (Fig. 1b and c). Thus, co-implanted MSCs may topically promote the settlement and earlier onset of tumor growth but may not affect the kinetics of tumor growth and progression.

Table I. Onset incidence of tumor formation (%) after subcutaneous inoculation.

Weeks	0	1	2	3	4	5	6
Group 1 (n=5)	0	0	0	0	60	80	100
Group 2 (n=5)	0	0	0	0	0	0	0
Group 3 (n=5)	0	40	60	80	100	100	100

Group 1, COS1NR ($5x10^6$ cells) alone; Group 2, MSC alone ($5x10^6$ cells); Group 3, COS1NR+MSC ($5x10^6$ cells each) in PBS inoculated into subcutaneous tissue of F344 rats.

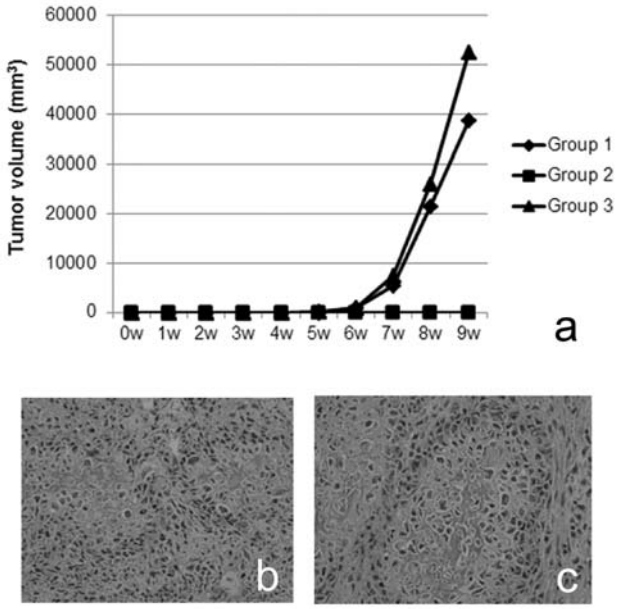

Figure 1. (a) Growth curves of subcutaneous tumors in each group. Group 1, COS1NR ($5x10^6$ cells) alone; Group 2, MSC ($5x10^6$ cells) alone; Group 3, COS1NR+MSC ($5x10^6$ cells each). (b) Histological appearance of subcutaneous tumors in Group 1 (H&E, x200). (c) Histological appearance of subcutaneous tumors in Group 3 (H&E, x200).

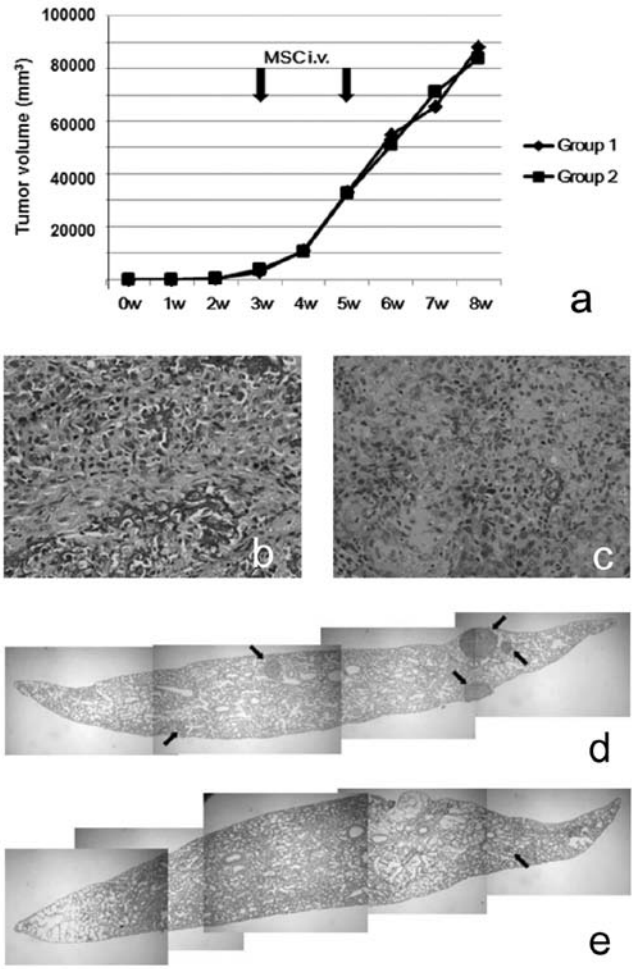

Figure 2. (a) Growth curves of subcutaneous tumors in each group. Group 1, COS1NR ($5x10^6$ cells) with intravenous injection of MSCs ($5x10^6$ cells) at weeks 3 and 5 (n=5); Group 2, COS1NR alone ($5x10^6$ cells) without MSCs injection (n=5); (b) Histological appearance of subcutaneous tumors in Group 1 (H&E, x200) (c) Histological appearance of subcutaneous tumors in Group 2 (H&E, x200). (d) Histological appearance of lung specimen from Group 1 (H&E, x40). (e) Histological appearance of lung specimen from Group 2 (H&E, x40). Black arrows indicate lung metastatic foci, significantly increased in Group 1.

Effect of intravenous injection of MSCs on metastatic potential in rat osteosarcoma model. Upon the effect of MSCs in earlier onset of tumor development, we examined whether circulated MSCs might have an impact on the development of metastasis in rat osteosarcoma model. After tumor implantation subcutaneously, MSCs were administered intravenously via the rat tail vein twice at weeks 3 and 5, then the growth of tumor and the formation of lung metastatic nodules were evaluated as described above at week 8. There were no statistically significant variation in tumor volume observed between the groups with and without intravenous injection of MSCs (Fig. 2a, 86149.9±50954.5 vs. 85462.5±46754.9 mm^3, p=0.98), neither were differences observed in histological appearance between these groups (Fig. 2b and c). However, the number of lung metastatic nodules was significantly increased in the group with MSCs injection compared to the group without MSCs (17.33±8.39 vs. 2.0±2.0, p=0.03, Fig. 2d and e), suggesting that the MSCs may promote tumor cell settlement and onset of metastatic nodule formation.

Effect of MSCs on migration of osteosarcoma cells in vitro. The results from cell migration assay using transwell chamber showed that the migration of COS1NR osteosarcoma cells was promoted by both conditioned medium from MSCs and co-culturing with MSCs (Fig. 3). The differences between COS1NR/DMEM-10%FBS and COS1NR/MSC medium or COS1NR/MSC co-culture were statistically significant (p=0.003 and p=0.002, respectively). MSCs might secrete some factors which contribute to the invasiveness of osteosarcoma cells at least in part of the process of tumor growth and metastasis observed in the rat *in vivo* model.

Pathway expression profiles in rat MSCs and osteosarcoma cells. Under the consideration from the results of exogenous MSCs enhancing the early onset of tumor growth and the rate of lung metastasis *in vivo*, we performed pathway analysis by gene expression profile to investigate the differences of gene expression lacking in osteosarcoma cells and highly expressed in MSCs, being possibly involved in tumor engraftment, migra-

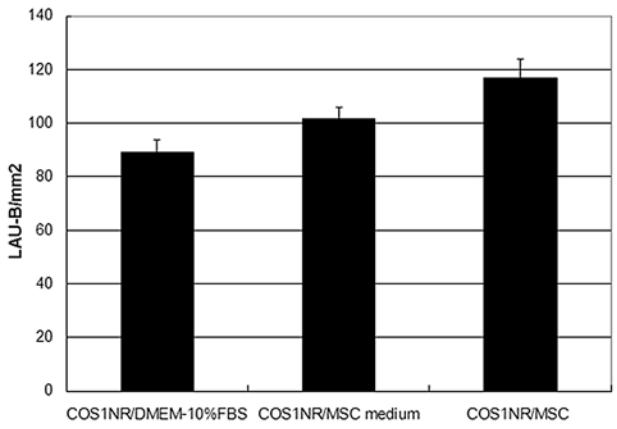

Figure 3. Evaluation of *in vitro* cell migration potential of rat osteosarcoma cells by Cytoselect 96-Well Cell Invasion Assay. Migration potential was elevated under presence of conditioned medium from cultured MSCs or co-culture of MSCs.

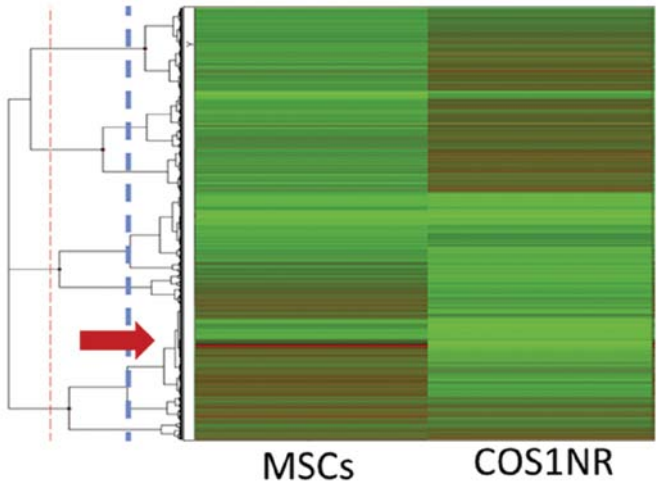

Figure 4. Gene expression profiling by Agilent array analysis comparing MSCs and osteosarcoma COS1NR cells. Red arrow indicates the cluster of genes, including focal adhesion, cytokine-cytokine receptor and extracellular matrix-receptor pathways, which were significantly up-regulated (thick red band) in MSCs compared to COS1NR.

tion and invasion. Results identified that genes involved in focal adhesion, cytokine-cytokine receptor and extracellular matrix-receptor pathways were highly significantly expressed in MSCs compared to osteosarcoma cells (Fig. 4). Among these, CAMs (ICAM and VCAM) were significantly overexpressed in MSCs compared to osteosarcoma cells, while integrins, especially β4 as well as α1 were strongly expressed in osteosarcoma cells. CXCL12 and CXCR4 axis was elevated in MSCs, but CXCR7, another receptor for CXCL12, was highly expressed in osteosarcoma cells compared to MSCs. In addition, matrix metalloproteinases (MMPs)-2 and 9 were elevated in MSCs compared to osteosarcoma cells (Table II and Fig. 5). These results might provide the clue how MSCs mediate the interaction with osteosarcoma cells to accelerate their invasiveness as well as adhesion and migration that possibly resulted in increased metastatic incidence and tumor progression. Further study will reveal the detailed mechanisms and role of MSCs involvement in the tumor microenvironment of sarcomas.

Table II. Agilent array gScale signal intensity.

Gene	MSC	COS1NR
ICAM/CD54	50432.9	1091.1
VCAM/CD106	77204.4	264.81
Integrin α-1	15110.2	14163.1
Integrin β-1	10724.8	6737.2
Integrin β-4	2391.1	5523.2
CXCL12	104134.4	15695.4
CXCR4	10006.1	17.4
CXCR7	1487.9	4673.8
MMP-2	83605.6	546.0
MMP-9	5889.2	3213.7

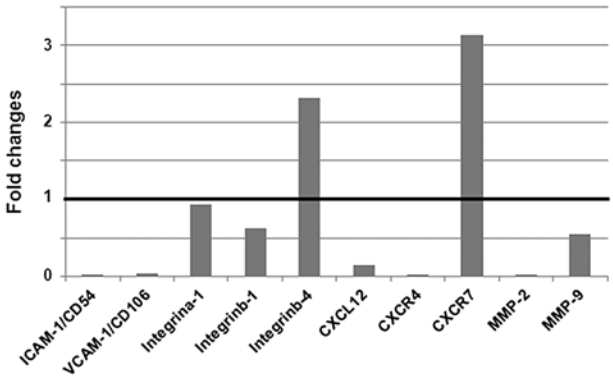

Figure 5. Fold changes of gScale signals for representative genes involved in adhesion, cytokine-cytokine receptors and extracellular matrix pathways. Integrin β4 (2.3-fold) and CXCR7 (3-fold) are highly expressed in osteosarcoma cells compared to MSCs, while others are strongly expressed in MSCs, especially CAMs, CXCL12 and MMPs.

Discussion

Substantial evidence has accumulated regarding the roles of MSCs and MSC-derived tumor stromal cells such as cancer associated fibroblasts (CAF), implicated in important aspects of tumor biology such as tumor growth, angiogenesis, and metastasis (31,32). The aim of the current study was to investigate the role of MSCs in the growth and development of osteosarcoma in a rat model.

The results demonstrated that the increased incidence of tumor engraftment and enhanced earlier onset of growth were observed in rats co-inoculated with MSCs-tumor cell mixture compared to tumor cells alone, and intravenous injection of MSCs significantly enhanced the incidence of pulmonary metastasis. The incidence of tumor formation from inoculation of MSCs and tumor cell mixture ($5x10^6$ each) was almost equal to our previous result from inoculation of $1x10^7$ of osteosarcoma cells (data not shown), suggesting a very early effect of MSCs on tumor engraftment in microenvironment of both subcutis and lung tissues in our rat model. There are several possible explanations for these results. Firstly, it is known that MSCs are able to produce various growth factors that stimulate angio-

genesis, and in response to these factors, endothelial precursors may be recruited into tumor neovascularization process (33,34). Therefore, it might be possible that factors secreted by MSCs merged with osteosarcoma cells might induce angiogenesis, which led to enhanced blood supply and growth of the tumor. Secondly, factors secreted by exogenous MSCs may directly stimulate the growth of tumor cells (35).

The possible reason for higher rate of pulmonary metastasis could be that factors secreted by MSCs induced osteosarcoma cells to ride on the route to pulmonary metastasis. The exogenous MSCs administered through caudal vein have been demonstrated in bone marrow, spleen, liver and lungs at 20-24 h after infusion, and this migration of MSCs occur spontaneously departing from bone marrow *in vivo* (36). Infused exogenous MSCs also preferentially homed to tumor sites and were involved in the formation of tumor stroma (14). In addition, the number of circulating MSCs was increased under some pathological conditions such as muscle injuries and burns. This increased number of circulating MSCs was also observed in patients with breast cancer and bone sarcomas (37,38). Therefore, it would be possible that migrating MSCs in the lung could merge with metastatic tumor stroma and promote pulmonary metastasis development (18). Further study regarding the kinetics of MSCs *in vivo* such as fluorescence labeling will be required to elucidate the role of MSCs in the metastatic process.

Immunosuppressive properties of MSCs could be another factor in part to promote the tumor developments. MSCs have been shown to suppress the lymphocyte proliferative response in a broad way regarding the types of stimulation and of lymphocyte population (16,39). A number of mechanisms have been reported for these effects, such as secretion of anti-inflammatory factors (40), modulation of the function of major immune cell population (41), cell cycle arrest in the G0/G1 phase on B lymphocyte (42), modulation of development and function of cytotoxic T cells and dendritic cells (43,44), inhibition of interferon-γ production by activated natural killer cells (45). These mechanisms could be the clue to explain how tumor cells evade the immune system. One hypothesis is that tumor cells can harness MSCs that manipulate the immune system through several ways described above, and eventually evade the immune attack (3).

CAMs (ICAM and VCAM) and integrins have been shown to be critical in tumor cell interaction with the microenvironment. ICAM-1 has also been recognized as one of the mesenchymal stem cell-associated markers and plays a role in cell-cell interaction with biding to integrins (46). Integrins constitute an important family of cell adhesion receptors responsible for mediating interaction between cells and the extracellular matrix (47). To date, 25 integrin heterodimers have been identified to regulate the key biological processes such as adhesion, signaling, migration, proliferation, angiogenesis and metastasis (47). Among these, α6β4, originally identified as a tumor-associated antigen, has been shown to be particularly relevant in invasive and metastatic carcinomas (48), and β4 integrin is implicated in cancer progression, correlating with poor prognosis in osteosarcoma (49). Since tumor cells are less likely to exist as a single cell suspension *in vivo*, it has been shown that multicellular clusters of tumor and stroma cells rather than single cells penetrated the microvasculature (50). The current study suggests that MSCs might participate in forming these clusters through their adhesion molecules interacting with the receptors on tumor cells, and possibly assist the migration and invasion.

Chemokine and chemokine receptor interaction plays an important role in tumor progression and development (51,51). Many human cancers have a complex chemokine network that influences the leukocyte infiltration, tumor cell growth, survival, migration and angiogenesis. Thus, we focused on chemokine pathways for the analysis of gene expression profiles on Agilent array, in particular, the chemokine CXCL12 (SDF-1) and its receptor CXCR4 and CXCR7, which promote growth of primary tumors and progression to metastasis in several cancer types. The results showed that CXCL12/CXCR4 axis was highly expressed in MSCs compared to osteosarcoma cells. However, another CXCL12 receptor, CXCR7 was highly expressed in osteosarcoma cells rather than in MSCs. Several reports indicated that CXCL12/CXCR7 axis promoted tumor growth *in vivo* and enhanced experimental metastasis in breast and lung cancer cells independently upon CXCL12/CXCR4 axis (53,54). Thus, the current results suggest that CXCL12/CXCR7 axis might be involved in osteosarcoma cell-MSCs interaction rather than CXCL12/CXCR4 axis for tumor development.

Additionally, increased tumor metastasis by injection of MSCs was possibly achieved in part by elevating the basement membrane degrading enzymes, MMP-2 and 9 in MSCs.

These results suggest that MSCs might compensate the ability of osteosarcoma cells with their dominantly activated pathways to support tumor development. Further investigation on the details of chemokine interaction and involvement of other molecules between osteosarcoma cells and MSCs will be required in the future.

Recently, cancer stem cell hypothesis has emerged and several reports described the presence of stem-like cells in sarcomas as well, contributing to tumor progression such as metastasis and drug resistance (55). Stem cells in normal condition require the 'niche', a specialized physiological microenvironment in which stem cells reside, playing a crucial role in the maintenance of stem cell characteristics such as pluripotency and self-renewal. The stem cell niche is composed of a group of cells providing a physical anchoring site for stem cells with the interaction of adhesion molecules between stem cells and niche cells or extracellular matrices. Some functional environments, namely 'cancer stem cell niche', a counterpart of normal stem cell niche, may exist and support cancer stem cells like normal stem cells (56). Although even the niche for MSCs is still unclear and if MSCs are the cell-of-origin for sarcoma cells, the niche for sarcoma stem cells, i.e. malignant transformed MSCs, is far more unclear. MSCs could be candidates for a resource of cancer/sarcoma stem cell niche, possibly playing an important role in orchestrating the tumor microenvironment through angiogenesis, modulation of both immune system and tumor stromal architecture. Understanding the interaction between MSCs and their niche will lead to understanding their counterpart, the interaction between sarcoma stem cells and their niche, and eventually to future treatment strategy targeting them or their interaction.

In conclusion, the results from the current study indicate that the administration of exogenous MSCs may promote tumor engraftment and early onset of growth *in vivo* as well as metastasis, possibly through the modulation of intercellular signaling

pathways between tumor-tumor cells and tumor-stromal cells in the tumor microenvironment. Further study will elucidate the role of MSCs as a possible component of tumor microenvironment or 'cancer stem cell niche' in sarcomas.

Acknowledgements

Authors declare no conflict of interest. This study was supported by a Grant-in-Aid for Scientific Research (No. 20591765 to K.H.) from Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. Paget S: The distribution of secondary growths in cancer of the breast. Lancet 1: 571-573, 1889.
2. Albini A and Sporn MB: The tumour microenvironment as a target for chemoprevention. Nat Rev Cancer 7: 139-147, 2007.
3. Yen BL and Yen M-L: Mesenchymal stem cells and cancer-for better or for worse? J Cancer Mol 4: 5-9, 2008.
4. Friednstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF and Keikiss-Borok IV: Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues, cloning in vitro and retransplantation in vivo. Transplantation 17: 331-340, 1974.
5. Prockop DJ: Marrow stromal cells as stem cells for non-hematopoietic tissues. Science 276: 71-74, 1997.
6. Brooke G, Cook M, Blair C, Han R: Heazelwood C, Jones B, Kambouris M, Kollar K, McTaggart S, Pelekanos R, Rice A, Rosetti T and Atkinson K: Therapeutic applications of mesenchymal stromal cells. Semin Cell Dev Biol 18: 846-858, 2007.
7. Pittenger MF, Machay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S and Marshak DR: Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-147, 1999.
8. Jorgensen C, Noel D, Apparailly F and Sany J: Stem cells for repair off cartilage and bone: the next challenge in osteoarthritis and rheumatoid arthritis. Ann Rheum Dis 60: 305, 2001.
9. Nauta AJ and Fibbe WE: Immunomodulatory properties of mesenchymal stromal cells. Blood 110: 3499-3506, 2007.
10. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fibbe W and Ringden O: Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase-II study. Lancet 371: 1579-1586, 2008.
11. Devine SM, Bartholomew AM, Mahmud N, Nelson M, Patil S, Hardy W, Sturgeon C, Hewit T, Chung T, Stock W, Sher D, Weissman S, Ferrer K, Mosca J, Deans R, Moseley A and Hoffman R: Mesenchymal stem cells are capable of homing to the bone marrow of nonihuman primates following systemic infusion. Exp Hemtol 29: 244-255, 2001.
12. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N and Phinney DG: Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc Natl Acad Sci USA 100: 8407-8411, 2003.
13. Chamberlain G, Fox J, Ashton B and Middleton J: Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 25: 2739-2749, 2007.
14. Studeny M, Marini FC, Champlin RE, Zompetta C, Fidler IJ and Andreeff M: Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. Cancer Res 62: 3603-3608, 2002.
15. Dwyer RM, Potter-Beirne SM, Harrington KA, Lowery AJ, Hennessy E, Murphy JM, Barry FP, O'Brien T and Kerin MJ: Monocyte chemotactic protein-1 secreted by primary breast tumors stimulates migration of mesenchymal stem cells. Clin Cancer Res 13: 5020-5027, 2007.
16. Djouad F, Plence P, Bony C, Tropel P, Apparailly F, Sany J, Noel D and Jorgensen C: Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood 102: 3837-3844, 2003.
17. Djouad F, Bony C, Apparailly F, Louis-Plence P, Jorgensen C and Noel D: Earlier onset of syngenic tumors in the presence of mesenchyaml stem cells. Transplantation 82: 1060-1066, 2006.
18. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R and Weinberg RA: Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 449: 557-563, 2007.
19. Beckermann BM, Kallifatiditis G, Groth A, Frommhold D, Apel A, Mattern J, Salnikov AV, Moldenhauer G, Wagner W, Diehlmann A, Saffrich R, Schubert M, Ho AD, Giese N, Buchler MW, Friess H, Buchler P and Herr I: VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. Br J Cancer 99: 622-631, 2008.
20. Annabi B, Naud E, Lee YT, Eliopoulos N and Galipeau J: Vascular progenitors derived from murine bone marrow stromal cells are regulated by fibroblast growth factor and are avidly recruited by vascularizing tumors. J Cell Biochem 91: 1146-1158, 2004.
21. Hung SC, Deng WP, Yang WK, Kiu RS, Lee CC, Su TC, Lin RJ, Yang DM, Chang CW, Chen WH, Wei HJ and Gelovani JG: Mesenchymal stem cell targeting of microscopic tumors and tumor stroma development monitored by noninvasive in vivo positron emission tomography imaging. Clin Cancer Res 11: 7749-7756, 2005.
22. Mackall CL, Meltzer PS and Helman LJ: Focus on sarcomas. Cancer Cell 2: 175-178, 2002.
23. Li Q, Hisha H, Takaki T, Adachi Y, Li M, Song C, Feng W, Okazaki S, Mizokami T, Kato J, Inaba M, Hosaka N, Maki M and Ikehara S: Transformation potential of bone marrow stromal cells into undifferentiated high-grade pleomorphic sarcoma. J Cancer Res Clin Oncol 136: 829-838, 2010.
24. Mohseny AB, Szuhai K, Romeo S, Buddingh EP, Briaire-de Bruijn I, De Jong D, van Pel M, Cleton-Jansen AM and Hogendoorn PC: Osteosarcoma originates from mesenchymal stem cells in consequence of aneuploidization and genomic loss of Cdkn2. J Pathol 219: 294-305, 2009.
25. Li N, Yang R, Zhang W, Dorfman H, Rao P and Gorlick R: Genetically transforming human mesenchymal stem cells to sarcomas: changes in cellular phenotype and multilineage differentiation potential. Cancer 115: 4795-4806, 2009.
26. Xu W-T, Bian Z-Y, Fan Q-M, Li G and Tang T-T: Human mesenchymal stem cells (hMSCs) target osteosarcoma and promote its growth and pulmonary metastasis. Cancer Lett 281: 32-41, 2009.
27. Honoki K, Tsutsumi M, Miyauchi Y, Mii Y, Tsujiuchi T, Morishita T, Miura S, Aoki M, Kobayashi E, Tamai S and Konishi Y: Increased expression of nucleoside diphosphate kinase/nm23 and c-Ha-ras mRNA is associated with spontaneous lung metastasis in rat-transplantable osteosarcomas. Cancer Res 53: 5038-5042, 1993.
28. Honoki K, Mori T, Tsutsumi M, Tsujiuchi T, Kido A, Morishita T, Miyauchi Y, Dohi Y, Mii Y, Tamai S and Konishi Y: Heterogeneous pattern of gene expression in cloned cell lines established from a rat transplantable osteosarcoma lung metastatic nodule. Cancer Lett 127: 221-228, 1998.
29. Tohma Y, Ohgushi H, Morishita T, Dohi Y, Tadokoro M, Tanaka Y and Takakura Y: Bone marrow-derived mesenchymal cells can rescue osteogenic capacity of devitalized autologous bone. J Tissue Eng Regen Med 2: 61-68, 2008.
30. Luu HH, Kang Q, Park JK, Si W, Luo Q, Jiang W, Yin H, Montag AG, Simon MA, Peabody TD, Haydon RC, Rinker-Schaeffer CW and He TC: An orthotopic model of human osteosarcoma growth and spontaneous pulmonary metastasis. Clin Exp Metastastasis 22: 319-329, 2005.
31. Mueller MM and Fusenig NE: Friends or foes - bipolar effects of the tumour stroma in cancer. Nat Rev Cancer 11: 839-849, 2004.
32. Roorda BD, ter Elst A, Kamps WA and De Bont ES: Bone marrow-derived cells and tumor growth: contribution of bone marrow-derived cells to tumor microenvironments with special focus on mesenchymal stem cells. Crit Rev Oncol Hematol 3: 187-198, 2009.
33. Relf M, Lejeune S, Scott PA, Fox S, Smith K, Leek R, Moghaddam A, Whitehouse R, Bicknell R and Harris AL: Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 57: 963-969, 1997.
34. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH and Verfaillie CM: Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest 109: 337-346, 2002.
35. Roorda BD, ter Elst A, Meeuwsen-de Boer TGJ, Kamps WA and De Bont ESJM: Mesenchymal stem cells contribute to tumor cell proliferation by direct cell-cell contact interactions. Cancer Invest Early Online: 1-9, 2010.

36. Kyriakou C, Rabin N, Pizzey A, Nathwani A and Yong K: Factors that influence short-term homing ofhuman bne marrow-derived mesenchymal stem cells in a xenogenic animal model. Hematologica 93: 1457-1465, 2008.
37. Fernadez M, Simon V, Herrera G, Cao C, del Favero H and Minguell JJ: Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. Bone Marrow Transplant 20: 265-271, 1997.
38. Bian Z-Y, Li G, Gan Y-G, Hao Y-Q, Xu W-T and Tang T-T: Increased number of mesenchymal stem cell-like cells in peripheral blood of patients with bone sarcomas. Arch Med Res 40: 163-168, 2009.
39. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S and Gianni AM: Human bone marrow stroma cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 99: 3838-3843, 2002.
40. Rasmusson I: Immune modulation by mesenchymal stem cells. Exp Cell Res 312: 2169-2217, 2006.
41. Glennie S, Soeiro I, Dyson PJ, Lam EW-F and Dazziet F: Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood 105: 2821-2827, 2005.
42. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V and Uccelli A: Human mesenchymal stem cells modulate B-cell functions. Blood 107: 367-372, 2006.
43. Rasmusson I, Ringden O, Sundberg B and Le Blanc K: Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activates cytotoxic T lymphocytes or natural killer cells. Transplantation 76: 1208-1213, 2003.
44. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD and Mao N: Human mesencymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 105: 4120-4126, 2005.
45. Aggarwal S and Pittenger MF: Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105: 1815-1822, 2005.
46. Majumdar MK, Keane-Moore M, Buyaner D, Hardy WB, Moorman MA, McIntosh KR and Mosca JD: Characterization and functionality of cell surface molecules on human mesenchymal stem cells. J Biomed Sci 10: 228-241, 2003.
47. Hood JD and Cheresh DA: Role of integrins in cell invasion and migration. Nat Rev Cancer 2: 91-100, 2002.
48. Falcioni R, Sacchi A, Resau J and Kennel SJ: Monoclonal antibody to human carcinoma-associated protein complex: quantitation in normal and tumor tissue. Cancer Res 48: 816-821, 1988.
49. Wan X, Kim S-Y, Guenther LM, Mendoza A, Briggs J, Yeung C, Currier D, Zhang H, Mackall C, Li W-J, Tuan RS, Deyrup AT, Khanna C and Helman J: Beta4 integrin promotes osteosarcoma metastasis and interacts with ezrin. Oncogene 28: 3401-3411, 2009.
50. Simiantonaki N, Jayasinghe C and Kirkpatrick CJ: Differential endothelial CAM-expression after stimulation with supernatants of LPS-and cytokine-stimulated HT-29 and ST-ML-12 tumor cells growing as monolayer cultures and multicellular spheroids. Anticancer Res 22: 2641-2649, 2002.
51. Balkwill F: Cancer and chemokine network. Nat Rev Cancer 4: 540-550, 2004.
52. Luker K and Luker G: Functions of CXCL12 and CXCR4 in breast cancer. Cancer Lett 238: 30-40, 2006.
53. Burns JM, Summers BC, Wang Y, Melikian A, Berahovich R, Miao Z, Penfold MET, Sunshine MJ, Littman DR, Kuo CJ, Wei K, McMaster BE, Wright K, Howard MC and Shall TJ: A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. J Exp Med 203: 2201-2213, 2006.
54. Miao Z, Luker KE, Summers BC, Berahovich R, Bhojan MS, Rehemtula A, Kleer CG, Essner JJ, Nasevicius A, Luker GD, Howard MC and Schall TJ: CXCR7 (RDC1) promotes breast and lung tumor growth in vivo and is expressed on tumor-associated vasculature. Proc Natl Acad Sci USA 104: 15735-15740, 2007.
55. Honoki K: Do stem-like cells play a role in drug resistance of sarcomas? Expert Rev Anticancer Ther 10: 261-270, 2010.
56. Liu A, Ginestier C, Ou SJ, Clouthier SG, Patel SH, Monville F, Korkaya H, Heath A, Dutcher J, Kleer CG, Jung Y, Dontu G, Taichman R and Wich MS: Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. Cancer Res 71: 614-624, 2011.