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Human Mesenchymal Stem Cells Migrate toward Colon Cancer Partially Regulated by HMGB1

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Abstract

Although Mesenchymal Stem Cells (MSC) has been explored as a new clinically relevant cell type to repair injured tissue, a number of studies have highlighted the important aspect of MSC therapy. Studies have shown that systemically administered MSCs migrate to sites of malignant tumor. The focus of this study was to identify the mechanism of migration of human MSCs into cancerous tissue. First, the effect of cultured medium from cancer cell lines on modulating the migration of MSCs was evaluated, using seven different human colon cancer cell lines. Interestingly, the secretion level of High Mobility Group Box 1 (HMGB1) protein from each cell line affected the migration capacity of MSCs. In addition, recombinant human HMGB1 increased MSC's migration capacity in a dose-dependent manner. Finally, 1×10^6 human MSCs were injected subcutaneously into mice (n=14) with colon cancer tumors that secreted high levels of HMGB1. Bioluminescence live image analysis showed that MSCs surrounded the tumors after injection into these mice through day 6. Immunohistochemical analysis using CD90 as a specific antibody revealed the existence of MSCs in and around the tumors as well as the secretion of local HMGB1 from the tumors detected by anti-HMGB1 antibody. These findings are critical in understanding the role of MSCs in development of solid tumors and further, they offer insight that may be useful in therapeutic application of MSCs in the treatment of malignant tumors.

Keywords: Mesenchymal stem cells; Malignant tumors; Immunohistochemical analysis; Stem cell therapy

Introduction

The migration capacity of Mesenchymal Stem Cells (MSC)s, which is considered to be a category of clinically relevant cell type, may be utilized for potential cell-based therapies. MSCs secrete an array of cytokines, chemokines, and growth factors [1], without the need for complicated culturing or handling techniques to yield clinically practical quantities of cells. The migration of MSCs into multiple inflamed or injured tissues has been studied in various models [2,3]. Indeed, isolated MSCs from bone marrow have been demonstrated to migrate toward inflamed tissues and affect via direct contact or in a paracrine fashion in the response of inflammatory cells such as macrophages [4,5], dendritic cells [6,7], and T-cells [3,8]. MSCs exist mostly in bone marrow niche [9], but also in various peripheral tissues including fat, salivary gland, tendon, skin, muscle, lung, and more recently, intestinal epithelium [10-16]. Interestingly, Da Silva Meirelles et al. and Crisan et al., suggested that perivascular lesions from a variety of tissues is a niche of MSCs, which may explain the biological features of multi-origination of MSCs [17,18]. However, the difference in the role of MSCs from these different niches such as bone marrow and perivascular lesions remains to be identified.

Recently, there has been heightened interest into the homing and migration capacity of MSCs into tumors. Since the process of tumor progression is highly related to inflammation, and since epithelial-mesenchymal transition is critical in cancer development [19], the role of MSCs in carcinogenesis has emerged as a new attractive concept in cancer therapy. Although ample experimental evidence exists supporting the therapeutic potential of MSCs, the mechanism of homing and recruitment of MSCs into tumors and their potential role in malignant tissue progression is still not well understood.

MSCs have been shown to promote tumor progression by immune modulation [20]. In contrast, some studies reported that MSCs have a suppressive effect on tumor development; e.g. via modification of Akt signaling [21]. The discrepancy between these results may be due to the use of different tissue sources, individual donor variability, and injection timing of MSCs. The expression of critical receptors such as Toll-Like Receptor (TLR) is variable at various time points during the treatment [22], which may also influence on the effect of MSCs to tumor progression. Whether MSCs support or suppress tumor, it is clear that systemically administered MSCs can be recruited by and migrate toward tumors [23,24]. These findings are important as they can be used as a basis for studies to explore the utilization of engineered MSCs as novel carriers for delivery of anti-tumor agents to cancerous tissue, for the development of tumor targeted therapies.

Several different mediators have been reported to be involved in the homing process of MSCs [25-27]. Some of these molecules such as growth factors, chemokines or cytokines have already been described as mediators that can regulate cell migration toward inflammatory

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sites, e.g. SDF-1, IFN-γ, CCL5/CCR5, CCR2, TNF-α and some other peptides [28-32]. Among these molecules, Meng et al. investigated whether High Mobility Group Box 1 (HMGB1) protein, considered to be a proinflammatory cytokine, could enhance the biological properties of human bone marrow MSCs [33]. HMGB1 is involved in several autocrine and/or paracrine feedback mechanisms resulting in positive enforcement of its expression, and that of its receptor, TLR4, has been reported to express on MSCs [34]. Since HMGB1 stimulates the expression of molecules correlated to vascular endothelial growth factor and platelet-derived growth factor signaling by inducing their secretion from tumor cells, HMGB1 has been identified as an important modulator of tumor angiogenesis [35].

In this study, we hypothesized that HMGB1 secreted from tumors is one of the key mediators that enhances recruitment and homing capacity of MSCs. We found that several colon tumor-derived cell lines secreted different amount of HMGB1 and MSCs could respond to it in a dose-dependent manner. In addition, we demonstrated the recruitment of MSCs into colon cancer *in vivo* using histological analysis and live cell tracking imaging system.

Materials and Methods

MSC culture

Human-derived MSCs, which were isolated from human bone marrow (kindly provided from Dr. Yumi Matsuzaki, Tokyo Medical University), were cultured in medium composed of D-MEM supplemented with 10% Fetal Bovine Serum (FBS), 0.1mM nonessential amino acids, 1 ng/ml of basic Fibroblast Growth Factor (bFGF), 100 U/ml penicillin, 100 μg/ml streptomycin. MSCs were passaged every 5-7 days and the medium was changed on every two days. MSCs were detached by 0.05% trypsin-Ethylete Diaminetetra Acetic Acid (EDTA), and were washed with PBS and resuspended in DMEM with 2%, 5% and 10% FBS for *in vitro* studies or in PBS for in vivo study.

Enzyme-Linked Immunosorbent Assay (ELISA) for HMGB1

Colon-cancer cell lines (WiDr, LoVo, SW480, SW867, HT15, HCT29, HCT115) as well as Human Umbilical Vein Endothelial Cells (HUVECs) and Neonatal Human Dermal Fibroblasts (NHDF) as controls, were incubated in serum free medium overnight, and the culture supernatants were analyzed for secreted HMGB1 protein using an ELISA kit (Chondrex Inc, WA, USA) according to the manufacture's instructions. Experiments were repeated three times.

Real time chemotaxis assay

Transwell chemotaxis assay was performed as described [36,37], with MSCs. EZ-Taxiscan chemotaxis assay was performed according to the manufacturer's protocol (GE Healthcare, GE health care Japan, Tokyo, Japan) using HMGB1 (Shino test, Tokyo, Japan) (0, 10, 50, 100, 500 ng/ml), and a variety of supernatant of colon-cancer cell lines (WiDr, LoVo, SW480, SW867, HT15, HCT29, and HCT115) as chemo-attractants. Experiments were repeated tree times.

Plasmid extraction and electroporation

Green Fluorescent Protein (GFP)-luciferase plasmid DNA (SV40-Luc) was kindly provided by Dr. Chikako Hara and Dr. Hideyuki Okano from the department of physiology, Keio University [38], and extracted from *E. coli* by means of NucleoBond® Xtra procedure kit (Macherey-Nagel, Düren, Germany). To transfect luciferase plasmid into MSCs with high efficiency, we used Amaxa™ Nucleofector™ technology (LONZA Japan, Tokyo, Japan) according to the manufacturer's protocol, which provides non-viral transfection technology with high viability after transfection. 4×10^5 of MSCs was applied for each transfection. Before injection, transfected MSCs were cultured for 7 days and the ratio of successfully transfected cells and cell viability were evaluated by detecting GFP-luminescence using a fluorescence microscope (Olympus IX71 Tokyo Japan).

Animals and Bioluminescence Analysis

NOD-SCID mice (5 week old, 17 ± 2 g) were obtained from CLEA Japan Inc. (Tokyo, Japan). One million cells of a human colon cancer cell line (SW480), which secreted high amount of HMGB1 in *in vitro* experiment, were injected subcutaneously into two sites at the back of the mice. Ten days later when tumors grew to approximately 8 mm in diameter, 1×10^6 MSCs transfected with dual-luciferase secretory gene as described, were administrated subcutaneously into the colon-cancer bearing mice (n=14). Anti human HMGB1 antibody (Abnova, Taipei, Taiwan) was administered 300 μg/body subcutaneously as described in previous study [39] as negative control (n=4). Subsequently, bioluminescence images of the mice were taken using the *in vivo* imaging system (IVIS® system) (Caliper Life Science, Hopkinton, MA, USA) by exposure for one minute. The bioluminescence color images were viewed using the image software overlay. Mice were monitored at 24 hours, 48 hours, and 6 days after the injection of the cells to obtain serial fluorescence images. All experimental procedures and protocols were approved by the Animal Ethics Committee of the Keio University, School of Medicine.

Histological analysis

For histological studies, spleen, liver, lung, kidney, brain, and tumor tissues were collected at 24 hours, 48 hours, and 6 days after the injection of MSCs (n=3 for each time point). Tissues were fixed in ice-cold 4% paraformaldehyde at 4°C in PBS buffer containing 0.1% sodium azide. Paraffin-embedding was performed by dehydration of blocks in a graded series of alcohol and immersion in a mixture of 6:4 low-temperature paraffin and paraffin oil at 40°C overnight. Section with 4 μm thickness were cut, dewaxed, rehydrated, and washed in double-distilled water for 5 minutes. Sections were stained with Hematoxylin and Eosin (H&E) and visualized by microscopy. Immunofluorescence staining was performed using anti-human CD90/Thy1 antibody [EPR3132] (Abcam Cambridge, UK) as a human MSC marker, and anti-human HMGB1 antibody (Abnova, Taipei, Taiwan). Alexa Fluor 594 goat anti-rabbit IgG (Invitrogen, Tokyo, Japan) was used as secondary antibody for rabbit anti-human CD90 antibody, and Alexa Fluor 488 goat anti-mouse IgG (Abcam, Cambridge, UK) was used as secondary antibody for mouse anti-human HMGB1 antibody. Auto-fluorescent cells including erythrocytes were distinguished from MSCs by using DAPI staining, which is a classic nuclear counterstain for immunofluorescence microscopy.

Statistic analysis

Comparisons between two groups were performed with Student's two-tailed t-test, and more than two groups were compared by Analysis of Variance (ANOVA) and considered significance at $P<0.05$ as described previously [40].

Results

Human colon cancer cell lines express various amount of HMGB1

It was reported that the migration capacity of MSC toward tumors

is partly regulated by HMGB1 mediated signaling [41]. We first measured secreted HMGB1 from 7 different human colon cancer cell lines (WiDr, LoVo, SW480, SW867, HT15, HCT29, and HCT115) as well as HUVEC and neonatal NHDF as controls by ELISA (Chondrex, WA, USA). HMGB1 was secreted at various concentrations from the different colon cancer cell lines (Figure 1a) as described in previous reports [42,43]. The highest secretion of HMGB1 was found in SW480 (127 ± 3.391 ng/ml), followed by LoVo (77.814 ± 9.979 ng/ml), and WiDr (68.132 ± 32.466 ng/ml), while the low level of secretion was detected from HT15 (16.409 ± 2.146 ng/ml), HT29 (45.439 ± 4.517 ng/ml), HCT116 (50.183 ± 5.057 ng/ml), and SW867 (50.537 ± 4.26 ng/ml) (Figure 1a). The secretion of HMGB1 from the human colon cancer cell lines was readily detectable by immunohistochemistry as shown in (Figure 1b). Based on these data, we chose SW480 as a high HMGB1-expressing cell line for the following experiments.

MSCs migrate toward supernatant of colon cancer cells

An optimal chemotaxis assay system, that allowed time-lapse video monitoring of cell behavior in silicon-coated micro-channels, was used to analyze the response of MSCs to supernatant from colon cancer cell lines as a chemo-attractant. Representative images of the cells migrating in the micro-channels are shown in (Figure 2a).

MSCs moved along the concentration gradient of the supernatant, showing that they could migrate directionally toward bioactive molecules secreted by cell lines derived from colon cancer. (Figure 2b) demonstrated that the plots in the velocity-direction graph of MSCs stimulated by the supernatant from SW480 (HMGB1 high expressing cell line) tended to align to the square on the above right which showed the cells moved toward the stimulant, compared to that of HCT15 (HMGB1 low expressing cell line) which plots were in the center of the graph. The migration of MSCs was analyzed as the ratio of the cells that migrated across the micro-channel to the total number of the cells in the assay area (Figure 2c). MSCs showed significantly stronger chemotaxis toward supernatant of SW480 (63.6 ± 19.9%), which expressed higher quantities of HMGB1 compared to HCT15 (22.1 ± 21.4%) which expressed relatively lower quantities of HMGB1 ($p<0.05$). In addition, we compared the velocity of the migration of MSCs between different supernatant as chemo-attractants. The results showed that the velocity of the cells was higher in the micro-channels with supernatant from cell lines expressing higher HMGB1 (e.g. SW480:0.00545 ± 0.0018 μm/sec) than in those containing supernatant from cell lines with a low expression level (e.g. HCT15:0.0434 ± 0.0012 μm/sec) ($p<0.05$) (Figures 2d).

MSCs migrate in a concentration gradient of HMGB1 in a dose-dependent manner

Since resent studies indicated that MSCs could respond to HMGB1 as a chemoatractant [33], we next investigated whether the migration capacity of MSCs was dependent on the concentration of HMGB1, by using recombinant human HMGB1 in the same chemotaxis assay system. We prepared different concentrations of HMGB1 (0, 10, 50, 100, and 500 ng/ml) based on the results of ELISA assay of supernatant from the cancer cell lines as previously described. As shown in (Figure 3a), the number of cells in the channel that moved from the starting compartment increased with the incubation time and MSCs migrated further toward the opposite compartment according to the concentration of HMGB1.

(Figure 3b) demonstrated that the plots in the velocity-direction graph of MSCs stimulated by HMGB1 in the concentration of 500 or 100 ng/ml tended to align to the square on the above right which showed the cells moved toward the stimulant, compared to that of 10 or 0 ng/ml which plots were in the center of the graph. MSCs were analyzed as the ratio of the cells that migrated across the micro-channel to the total number of the cells in the assay area (Figure 3c). The ratio of MSCs migrating toward compartments with higher concentration of HMGB1 was significantly higher (100 ng/ml: n= 57.0 ± 17.5%, 500 ng/ml: n= 57.2 ± 16.3%) than those with lower concentrations of HMGB1 (0 ng/ml: n= 8.9 ± 7.8%, 10 ng/ml: n= 28.5 ± 20.1%, 50 ng/ml: n= 29.9 ± 17.7%) (Figure 3c). In addition, MSCs moved with higher velocity when HMGB1 was administered in certain concentrations (e.g. 50 ng/ml: 0.00725 ± 0.0033 μm/sec, 100 ng/ml: 0.00727 ± 0.0029 μm/sec), compared to the control (0 ng/ml: 0.00585 ± 0.0035 μm/sec) ($p<0.05$), while the other concentrations of HMGB1 including 500ng/ml did not significantly increase their velocity compared to the control (Figure 3d).

MSCs migrate toward colon cancer expressing HMGB1

In order to assess the distribution of MSCs after the injection in human colon-cancer bearing mouse model, we used bioluminescence *in vivo* imaging system for cell tracking. MSCs were transfected using electroporation technique with GFP-luciferase expression vector. The

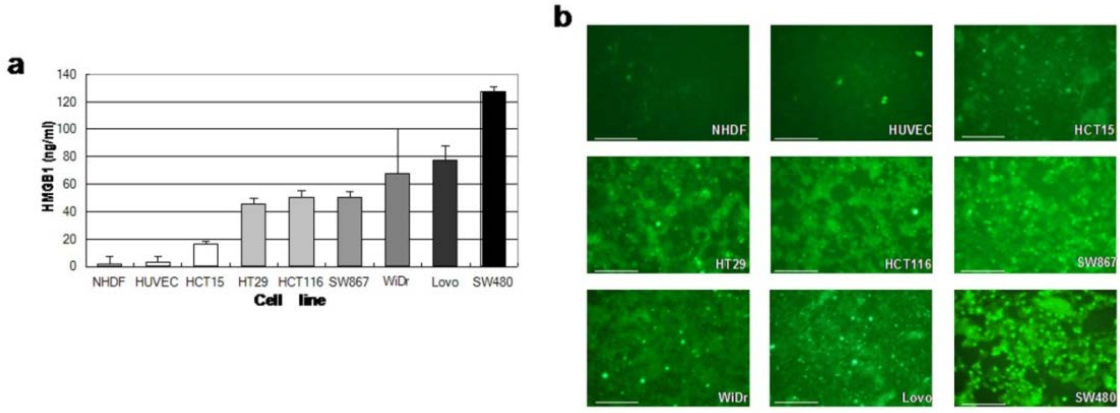

Figure 1: (a) The quantitative analysis of secreted HMGB1 protein from different seven colon-cancer cell line (HCT15, HT29, HCT116, SW867, WiDr, LoVo, SW480) Scale bars: 100 μm

(b) Fluorescence microscope image of cultured cells expressing HMGB1 as shown in. Scale bars: 100 μm

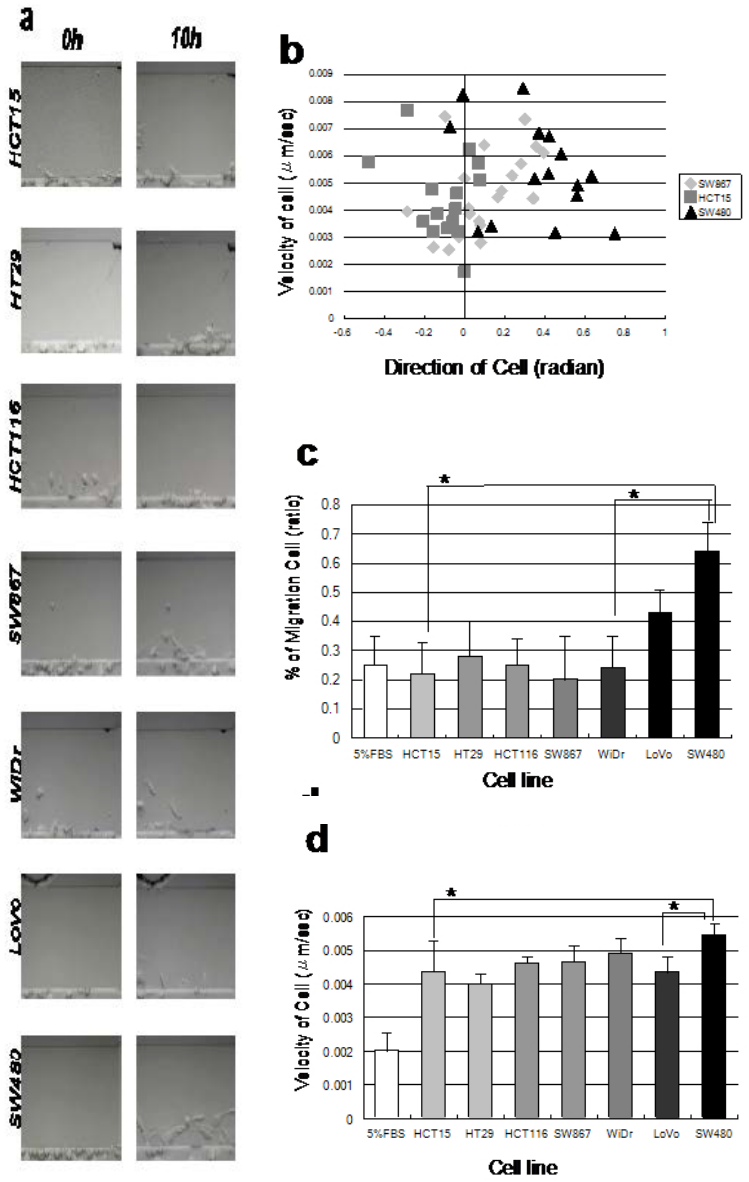

Figure 2: (a) MSCs were applied to the microchemotaxis chamber to see their migration capacity. Each supernatant of different seven colon-cancer cell lines was applied to the channel. Migration capacity of MSCs to the chemotaxis was analyzed as

(b) Velocity-direction plot graph

(c) Proportion of migrated cells

(d) The velocity of the cells. * $p<0.05$.

viability of the cells after the transfection was 80 ± 14% (Supplementary Figure 1). First, SW480 cells ($1x10^6$) (HMGB1 high expression cancer cell line) were injected subcutaneously into both sides of the lower back of NOD-SCID mice (n=14). Ten days after the injection of the cancer cells, $1x10^6$ MSCs were injected subcutaneously 5 to 10 mm to each tumor growth site (Figure 4a). As a negative control, 300 μg of anti-human HMGB1 antibody was administered subcutaneously into surrounding area of the tumors one day prior to the injection of MSCs. (Figure 4b) demonstrates the luciferase fluorescence of MSCs on a culture dish, detected by the same *in vivo* imaging system. It revealed the efficient transfection of the luciferase gene. (Figure 4c) represents the distribution of MSCs in a mouse 6 days after the injection. Luminescence signals in mice at each time point were clearly differentiated from surrounding tissues, and the higher "Tissue to Background Ratio" (TBK) value suggested that MSCs preferentially accumulated around tumor tissues and achieved their maximum value through day 6 after the injection. The signals on day 6 were localized at the peri-tumor areas and were significantly more intense than the signals on day 1, while the signals were detected almost in the same lesion at day 6 compared to day 1 when anti-HMGB1 antibody was administered (Figure 4d). Conversely, no luminescence signal could be observed in the tumor tissues on day 7 (data not shown).

Pathological evaluation of tumor and the surrounding tissue was

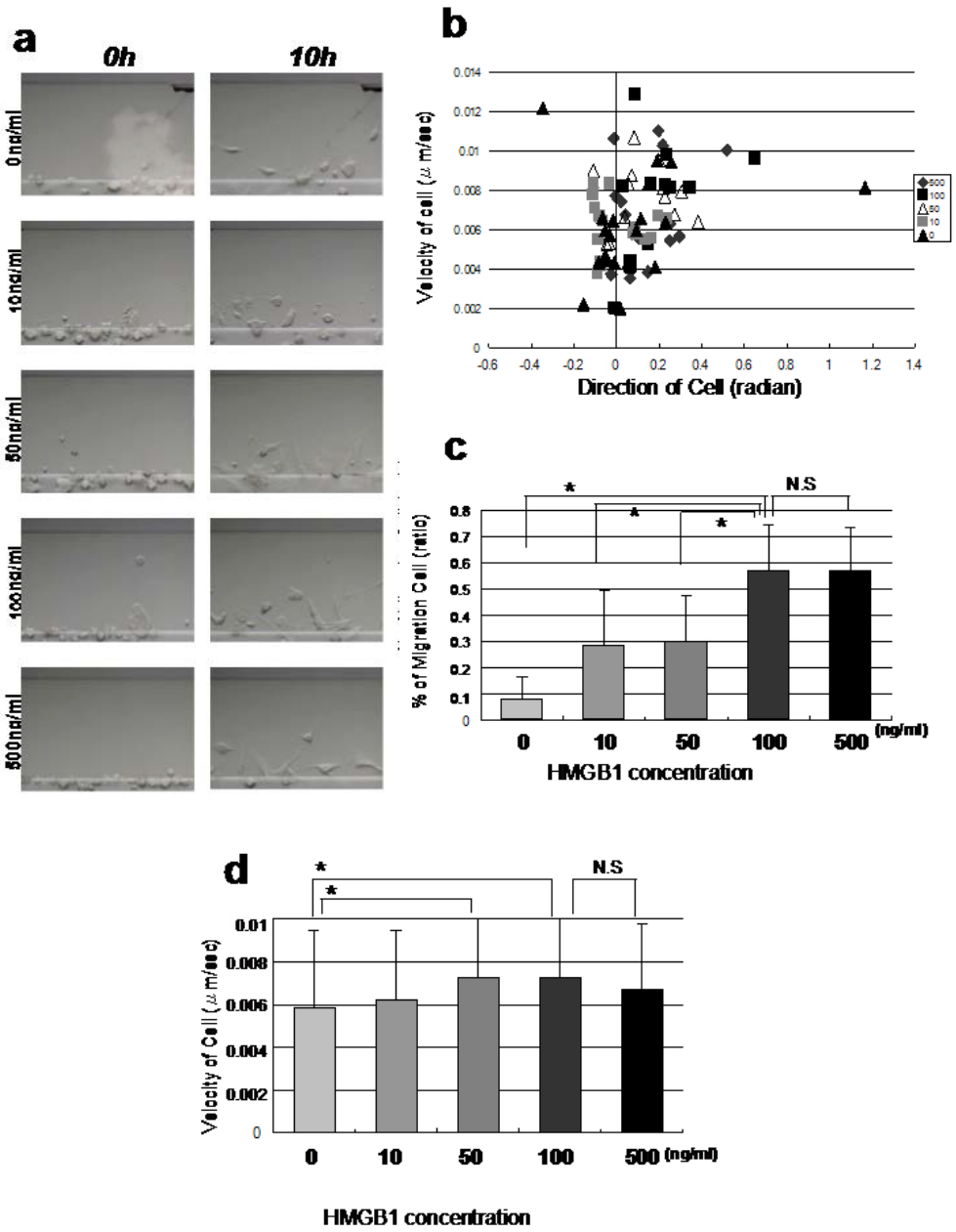

Figure 3: (a) MSCs were applied to the microchemotaxis chamber. Different concentrations of HMGB1 (0 ng/ml, 10 ng/ml, 50 ng/ml, 100 ng/ml, 500 ng/ml) were applied to the channel. Migration capacity of MSCs to the chemotaxis was analyzed as

(b) Velocity-direction plot graph

(c) Proportion of migrated cells

(d) The velocity of the cells. * $p<0.05$, N.S.: no significant

performed (Figure 4e). The excised organ tissues including liver, lung, spleen, kidney and heart were also analyzed (Supplementary Figure 2). Histological and immunohistochemical examination of central and peripheral areas (Figure 4e; upper row and middle row) of tumors was performed by H&E and immunofluorescent staining using anti-human CD90/Thy1 antibody, and anti-human HMGB1 antibody. Human CD90 was detected in tumor tissues obtained on day 6 after the administration of MSCs both in the parenchyma and the surrounding area of the tumor where HMGB1 secretion was also obtained in the tissues; however, MSCs were rarely detected in the tumor from mice administered anti-HMGB1 antibody where the binding of the administered antibody was detected by secondary antibody (Figure 4e; lower row). In addition, MSCs were not found in the other organs, in which no signals were detected with HMGB1 antibody (Supplemental Figure 2). In addition, tumor size was evaluated through day 14 and no significant difference was detected between mice with and without the injection of MSCs (data not shown).

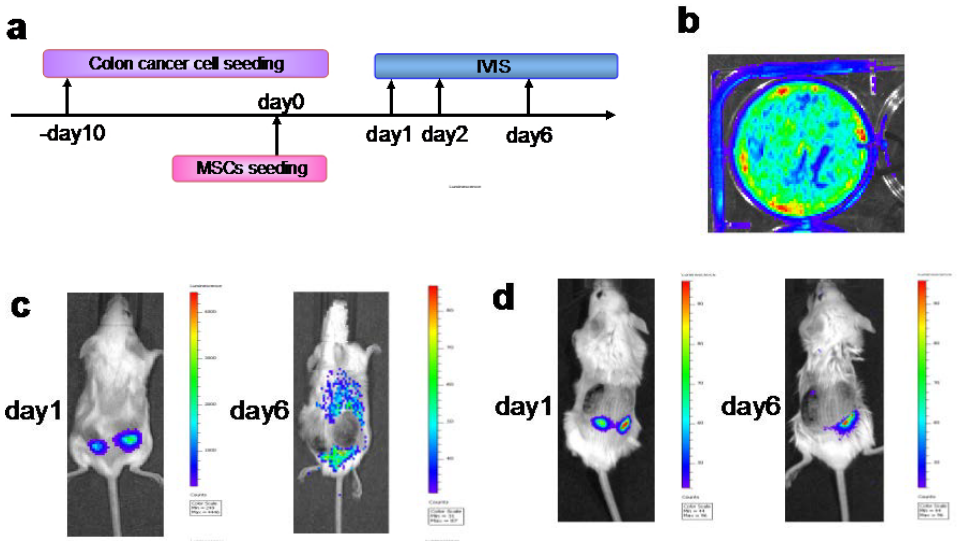

Figure 4: (a) The time course of the *in vivo* experiment.

(b) Representative image of luciferase expression of MSCs 24 hours after the transfection.

(c) *In vivo* fluorescence images of injected MSCs in colon-cancer mouse model at day1, day6.

(d) *In vivo* fluorescence images of injected MSCs in colon-cancer mouse model with the administration of anti-human HMGB1 antibody at day1, day 6.

(e) CD90 (MSCs) and HMGB1 was detected in the center of tumor (upper row) and peri-tumor (middle row) as arrows, while CD90 was not detected in the tumor from mice administered the antibody (lower row). Scale bars: 100 μm

Discussion

The previous reports [42,44] have led to a great deal of attention in the role and function of MSCs in tumors. Among these, recent study indicated that colon cancer is one of the target tumors which can recruit MSCs by secreting bioactive molecules [45,46]. The result of this study also indicated that MSCs could respond to factors secreted from colon cancer cells and these factors increased their capacity of migration, demonstrated by the channel device experiments where cultured medium from cancer cells was used as a stimulator.

The tropism of MSCs for tumors raised wide interest regarding their potential as delivery vehicles of anti-cancer agents. Indeed, several reports described the feasibility of using these cells as anti-cancer delivery vehicles since they secrete different anti-cancer molecules such as Tissue Necrosis Factor (TNF), TNF Related Apoptosis Inducing Ligand (TRAIL) or interferon (IFN)−β by gene modulation. These studies showed sufficient effect in suppressing tumor progression [10,23,41]. However, Karnoub et al. suggested that MSCs have a supportive effect on tumor progression showing that co-injection of MSCs with breast cancer cell-line led to a higher degree of metastasis, but this effect was not significant in local tumor growth [20]. Also, the supportive effect on tumor growth by MSCs has been reported in different cancers such as colon cancer, lymphoma, and melanomas [47-49]. In contrast, several reports showed that MSCs may have a suppressive role in tumor development via p38 mitogen-activated protein kinases (MAPK) or by cell fusion [50,51]. Also, different types of tumors such as liver cancer, breast cancer, leukemia, and pancreatic cancer have been used to show their tumor suppressive effect [52-55]. On the other hand, Torsvik et al. suggested that the cross-contamination of MSCs with tumor cells could enhance their tumor supportive behavior [56]. Interestingly, Klopp et al. suggested that the increased tumor mass observed in these reports could be related to increased proliferation of MSCs in the tumor [57]. In this study, the difference in tumor growth was not observed between mice injected or not injected MSCs through day 14 after cancer cell injection. But the animal model itself could affect to these response of the tumor to MSCs as the severity of immunosuppression. Further study is needed to clarify the biological effect of MSCs on tumor progression.

The response of MSCs to different biological stimulators has been shown in *in vitro* studies [32,58]. However, it is controversial whether systemically administrated MSCs can home to a target tissue and migrate into it. Some reports demonstrated that MSCs migrate towards inflammatory tissue [58] as well as cancerous tissue and may potentially be used for therapeutic purposes [49,59]; however, others showed that most of the cells were trapped in lymph nodes or the lungs and disappeared soon after injection [60]. This discrepancy could be partly explained by the difference of cell source such as bone-marrow or fat tissue and whether the cells were cultured or not cultured after isolation [61]. The MSCs used in this study were isolated from human bone-marrow and cultured for least 5 to 9 passages. Thus, the results could be different from studies that used MSCs derived from other tissues, and non-cultured primary MSCs. Also the mechanism of their behavior is unclear since MSCs can secrete an array of bioactive molecules in response to the conditions in their environment [31] and change their secretome with time [62]. Among these molecules, MSCs have been reported to respond to several different stimulatory factors such as TNFα, IL-6, SDF1 or soluble TNF receptor [28,31,32,63] and to migrate faster toward inflammatory or cancerous tissues. Actually, MSCs have receptors for these stimulator molecules [64,65] and can identify the elevation of these factors and dramatically react to their presence in the environment [31]. However, the mechanism of this action is unclear.

Previous studies suggested that HMGB1 is one of the key modulators of tumor development and its receptor, the receptor for advanced glycation end products (RAGE) [55] or Toll Like Receptor4 (TLR4) [35], is expressed on variety of cancer cells [66-69] including colon cancer [69]. HMGB1 was reported to have a potential role in the regulation of

cancer autophagy and apoptosis as response to the administration of anti-cancer agent [42] as well as to regulate migration and sprouting of endothelial cells as angiogenesis in tumor progression [35]. Moreover, the blockade of its receptor resulted in suppression of tumor growth and metastasis [66]. Therefore, HMGB1 has been recently identified as a potential candidate for tumor prevention therapy. In this study, we demonstrated HMGB1 was secreted in different concentrations by different cancer cell lines detected by ELISA and immunofluorescence study of cultured cells. Interestingly, MSCs have been reported to express one of these receptors TLR4 [34] and might react to HMGB1 through the TLR4 signaling pathway, which leads to the activation of NFκB that is also reported in MSCs with various stimuli [31]. Indeed, in our experiment, the chemotaxis assay using recombinant HMGB1 in different concentrations to the channel device (described in Methods) revealed that HMGB1 could modulate the migration capacity of MSCs in dose dependent manner. The result indicated that HMGB1 is one of the stimulating factors for migration of MSCs and activates their migration toward target cancer cells.

Based on these results in *in vitro*, we hypothesized that locally transplanted MSCs may react to the local stimulus and that their reactivity is partially linked to the activation of HMGB1 signaling pathway. To prove the concept that allo-transplanted MSCs can migrate toward solid tumors via HMGB1 secretion from the tumor, which we demonstrated by *in vitro* chemotaxis assay, we used a mouse model of human colon cancer and administered MSCs with or without anti-HMGB1 antibody. Indeed, the results showed that locally injected human MSCs migrated toward tumor and were both detected in the parenchyma and in the surrounding area of the tumor, while they were rarely detected in the tumor from the mice administered anti-HMGB1 antibody. These results may support the notion that MSCs in a peripheral tissue niche can respond to a tumor, which may exist a short distance from their niche, and can migrate into it. HMGB1 may regulate this process as a stimulatory molecule secreted from the tumors through TLR4 expressed on MSCs, as previously described [22]. Also our study suggested that the blockage of HMGB1 by its antibody modulated the migration capacity of locally administered MSCs *in vivo*. In addition, human MSCs were detected in the center and also in the edge of tumors where large numbers of HMGB1 positive cells were observed histologically. Although this is not direct proof that HMGB1 was the factor that brought about the migration of MSCs, the activation of MSCs by HMGB1 may be partially responsible for the observed migration of MSCs toward tumors. However, further study is required to clarify the effect of RAGE which is one of the key receptors of HMGB1, which is generally not presented on MSCs.

In summary, this study demonstrated that human MSCs migrate toward and into tumors in partially response to HMGB1 secreted from tumors into the milieu. These findings will help to understand the role of MSCs for tumors showing one of the key mechanisms. Since MSCs are relatively easy to obtain and grow in the laboratory, we anticipate that this study may lead to new approaches of cell therapy using MSCs to control cancer development.

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