Journal of
Stem Cell Research & Therapy
ISSN: 2157-7633

Newman et al., J Stem Cell Res Ther 2011, S2
DOI: 10.4172/2157-7633.S2-007

Research Article Open Access

Migration of Human Fetal Bone Marrow-derived Mesenchymal Stem Cells: Possible Involvement of GPCR and MMPs

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Abstract

Previously, we have demonstrated that not all adult human bone marrow-derived mesenchymal stem cells can migrate efficiently towards tumor cells. In the current study, we attempt to address whether different samples of human fetal bone marrow-derived mesenchymal stem cells (hfMSC) also exhibit different migratory capacities toward tumors as was found to be the case with adult human MSC. Further, we are keen to explore the underlying mechanism of how hfMSC home to tumor cells. Using modified Boyden chamber assay, we demonstrated that hfMSC migrate at an efficiency comparable or better than the highly migratory adult MSC. Unlike the adult MSC, the extent of migration was not correlated to the expression and activity of MMP1. Rather, it appeared to be dependent in part on the PAR1 expression which may in turn be modulated by GPCR signal pathways. Additional evaluation will need to be done to further confirm the exact migratory mechanism. Nevertheless, hfMSC may potentially serve as equally efficient carriers of therapeutic genes to tumors.

Introduction

The field of stem cell biology is rapidly integrating as new clinical trials in the treatment of a variety of pathologies ranging from regenerative medicine such as the repair of spinal cord injury to attempts of employing stem cells as tumor-tracking missiles for cancer treatment is moving into the clinics. It has been speculated that within the next ten years, stem cell therapeutics will revolutionize the treatment of human diseases. In the context of mesenchymal stem cells (MSC), this can be attributed to their ability to differentiate into mesodermal phenotypes of adipocytes, osteoblasts, chondrocytes, myocytes and those of extra-mesodermal cell types including neural, pancreatic, and hepatocyte phenotypes [1,2]. Furthermore, MSC possesses inherent immunomodulatory properties allowing their use as allogeneic cell sources without the risk of rejection [3] and the ability to migrate to sites of injury or inflammation [4-6]. More importantly, the tropism of MSC for tumors have made them uniquely suited to function as cellular delivery vehicles for potential cancer gene therapy. They can be genetically engineered to carry different therapeutic agents including interferon-β [7,8] tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [9]; cytosine deaminase [10] and oncolytic viruses [11]. Despite the potential therapeutic potential, there remained unanswered questions regarding how MSC achieve homing and interstitial migration, which has been frequently associated to resemble how leukocytes are recruited to the sites of inflammation.

Stem cell trafficking can be defined as orientated and directed movement of cells toward a specific stimuli. The mobilization of stem cells from the site of origin to peripheral tissues via the circulation requires fundamental molecular pathways involving niche-specific de-adhesion of the progenitor cells, chemoattraction to guide progenitor cell homing, integrin activation followed by a directional cue given by immobilized chemokines to navigate through into the extravascular tissues [12,13]. Advancing the understanding of these receptors on MSC may enable enhanced recruitment and migration towards the target tissue of interest. We have previously demonstrated that not all adult human mesenchymal derived-MSC can migrate efficiently towards gliomas [14]. Through careful analysis of those highly migratory MSC versus minimally migratory MSC, we have shown that matrix metalloproteinase-1 (MMP1) and its receptor play an important role in the homing process of MSC toward tumors *in vitro* and *in vivo*. It is perhaps not surprising since MSC will require the action of proteolytic enzymes for the degradation of extracellular matrix during migration and the subsequent extravasation event [15-17]. Apart from MMP1, MMP2, MT1-MMP1 (membrane-type matrix metalloproteinase-1), and TIMP-2 have also been shown to be essential for the invasive capacity of human MSC [17,18]. As mentioned earlier, MSC migration has been postulated to be similar to those found in leukocytes and their progenitor cell line, the haematopoietic stem cells (HSC). Among the various factors that are found to play a role in the migration of leukocytes and HSC, the interaction of stromal derived factor (SDF-1) with its receptor, the seven-transmembrane GPCR (CXCR4) has been implicated as the primary axis governing haematopoietic stem cell homing and engraftment in the bone marrow post transplantation. However, only a small subpopulation of human MSC has been shown to express functional cell surface CXCR4 receptors [19,20].

The signaling mechanism by which CXCR4 promotes cell migration is not fully understood. It is known that CXCR4 is coupled to heterotrimeric G proteins of the Gi family. When the

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Received October 07, 2011; **Accepted** December 12, 2011; **Published** December 14, 2011

Citation: Newman JP, Yi TX, Chan JKY, Endaya B, Lam P (2011) Migration of Human Fetal Bone Marrow-derived Mesenchymal Stem Cells: Possible Involvement of GPCR and MMPs. J Stem Cell Res Ther S2:007. doi:10.4172/2157-7633.S2-007

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ligand is bound, the heterotrimeric Gi complex dissociates into its α and βγ subunits. The released Gαi subunit inhibits adenylyl cyclase and the Gγ dimer activates two major signaling enzymes, the phosphatidylinositol 3-kinase (PI3K) [21] and phospholipase Cβ [22]. This subsequently initiates the activation of downstream events such as calcium mobilization, actin polymerization and chemotaxis. Using a combination of dominant negative and RNA interference approaches, Tan and colleagues have demonstrated that the Gα13 protein mediates the activation of Rho by CXCR4 and that the functional activity of both Gα13 and Rho is required for directional cell migration in response to SDF-1 [23]. Gα13 also appeared to be a critical transducer for GPCR such as PAR1 which in turn associates with the DIX domain of Dishevelled (DVL) which could lead to β-catenin stabilization [24]. During the process of cell migration, each member of the Rho GTPase family (e.g RhoA, Rac, Cdc42) plays a distinct role [25]. The GTP-bound Gα13 can then activate RhoA through the regulator of G-protein signaling (RGS) -containing Rho GEFs, p115 Rho GEF, PDZ-Rho-GEF or LARG [26].

In the current study, we attempt to address whether different samples of bone marrow derived-human fetal MSC (hfMSC) exhibit different migratory capacities towards tumors as was found to be the case with adult human MSC [14]. Further, we are keen to explore the underlying mechanism of how hfMSC home to tumor cells. hfMSC are primitive MSC which can be derived from the bone marrow, liver, blood and other tissues [27,28].They can be readily isolated and expanded. In additional to their abilities to differentiate into different lineages, they also express pluripotency markers with longer telomeres and are highly proliferative [29]. Thus, given enough understanding of the tumor tropism of these cells, they may potentially serve as better carriers of therapeutic genes to tumors than adult MSC.

Materials and Methods

Ethics

Fetal tissue collection was approved by the Domain Specific Review Board of National University Hospital, Singapore in compliance with international guidelines regarding the use of fetal tissue for research [30]. Pregnant women gave separate written consent for the clinical procedure and for the use of fetal tissue for research purposes. Fetal tissues were collected after clinically-indicated termination of pregnancy, and gestational age was determined by crown-rump length measurement. In this study, four different samples was utilized

Cell culture

Isolation and characterization of human adult MSC, MSC-1 and MSC-8 were performed as previously described [31]. They were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 with 10% fetal bovine serum (FBS, Invitrogen) and ascorbic acid (Sigma-Aldrich). Bone marrow derived-human fetal MSC (hfMSC), S8, S27, S33 and S64 were isolated from the fetal femur collected after clinically indicated termination of Pregnancy as previously described [32]. Human fetal MSC are cultured in DMEM supplemented with 10% fetal bovine serum (FBS; Invitrogen Life technologies), penicillin (100U/ml; Invitrogen, Grand Island, NY, USA), streptomycin (100µg/ml; Invitrogen), and 2mM L-glutamine (Sigma-Aldrich, MO, USA). HuH-7 was obtained from Japanese Collection of Research Bioresources (JCRB cell bank, Osaka, Japan) and grown under standard tissue culture condition in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT), 2 mM L-glutamine, and penicillin/streptomycin (100 U/ml).

Collection of conditioned medium

To collect the conditioned medium from human hepatocellular carcinoma cells (HCC) HuH-7, $1x10^6$ of HuH-7 cells were seeded in complete medium in a T75 flask and incubated at 37°C with 5% CO_2. An empty T75 flask containing the complete medium was treated in a similar manner. Medium were harvested after 72 h and centrifugated at 3000 rpm at 4°C to remove cellular debris. Supernatant were then collected and stored at -80ºC.

In vitro migration assay

For determining the effect of exogenous anti-PAR1 blocking antibody on the in vitro migration of hfMSC, a Modified Boyden chamber assay was done as previously described [14]. In brief, $1x10^4$ human adult and fetal MSC cells were cultured in 24-well tissue culture inserts with an 8 µm pore size membrane (BD Biosciences) and allowed to migrate for 8 h. Anti-PAR1 (20µg/mL, Santa Cruz Biotechnology Inc., Santa Cruz, CA) and Mouse IgG_1 (20µg/mL, DakoCytomation, Denmark) antibodies were added to the cells prior to and during the migration assay. Migration of MSC across the membrane was determined by counting the number of propidium iodide - stained nuclei on the underside of the membrane at x200 magnification with Nikon Eclipse 90i upright microscope using image acquisition software (NIS-elements AR 3.00).

MMP1 and MMP2 ELISA

Quantitation of MMP1 protein expression was performed using Quantikine human pro-MMP1 ELISA kit (R&D Systems), quantitation of MMP1 activity was performed using the MMP1 Biotrak activity assay system (GE Healthcare UK Limited, Little Chalfont, Buckinghamshire, UK) and quantitation of MMP2 protein expression were performed using Quantikine human MMP2 ELISA kit (R&D Systems Inc., Minneapolis, http://www.rndsystems.com) according to manufacturer's suggestions.

Immunoblotting

Equal amounts of proteins extracted from frozen cell lysates were resolved by SDS-PAGE and electroblotted onto polyvinylidine difluoride membrane (Trans-Blot Transfer medium; Bio-Rad Laboratories). Membranes were blotted against mouse anti-PAR1 (1:100) and rabbit anti-MMP2 (1:750) for 2 h RT, rabbit anti-Gα12 (1:600) and rabbit anti-Gα13 (1:400) from Santa Cruz Biotechnology (Santa Cruz, CA), and mouse anti-RhoA (1:1000), rabbit anti-cdc42 (1:1000), rabbit anti-p115RhoGEF (1:1000), rabbit anti-Rac (1:1000) from Cell Signaling Technology (Danvers, MA), and mouse pan actin clone Ab5 (1:50 000) from Neomarker (Fremont, CA) at 4°C O/N. Following washing and incubation with either goat anti-rabbit or goat anti-mouse horseradish peroxidase conjugated secondary antibodies (1:20000) (DakoCytomation, Denmark), protein of interest were visualized with an enhanced chemiluminescence using Western Lightning chemiluminescent kit (Perkin-Elmer, MA).

Statistical analysis

Statistical analysis was performed using Microsoft Excel (Microsoft). Non-paired parametric data were compared with two tailed Student's t-test. P-value of <0.05 was considered statistically significant. In addition, linear regression test was performed using Microsoft Excel.

Results

In the present study, we ask whether human fetal mesenchymal stem cells may also be non-migratory as observed in our earlier studies with the adult MSC. Human fetal mesenchymal stem cells were characterized by their fibroblast-like spindle morphology, ability to adhere to plastic and their immunophenotypic characteristics. They are CD45-, CD34-, CD14-, and CD31-negative and are positive for SH2-; SH3-, and SH4. Under permission conditions, they can differentiate to osteogenic and adipogenic lineages [27,29]. Using a modified Boyden chamber assay, we showed that the representative hfMSC isolates, namely S8, S27, S33 and S64 exhibited greater migratory activities towards conditioned medium derived from human hepatocellular carcinoma (HCC) when compared to the representative low migratory adult MSC isolate, MSC-8 (Figure 1). In particular, S8 migrated even better than MSC-1, which represents one of the highest migratory adult MSC isolates. We have characterized the migratory activities of another six hfMSC isolates, all of these cells are migrating better than the non-migratory adult MSC (i.e. higher than 50% when normalized to MSC-1) under similar experimental conditions (Table 1). Unlike the adult MSC, we did not observe any hfMSC that migrated poorly towards conditioned medium derived from tumor cells, suggesting that hfMSC highly migratory towards tumor cells.

Next, the total and activities of MMP1 protein expression was quantified using an ELISA assay. All migratory activities were normalized to MSC-1 as shown in Figs 2A and 2B. However, we did not observe a correlation in the expression and activities of MMP1 when compared to the migratory activities of hfMSC presented in Figure 1. The correlation coefficient is 0.6 for S8; 0.5 for S27; 0.8 for S33 and 0.5 for S64. Taken together, our results showed that hfMSC migration may be independent of MMP1. Previously, we have demonstrated that the extent of MSC migration in adult MSC is dependent on the level of MMP1 and its interaction with its cognate receptor, PAR1 [14]. To further elucidate the possible role of MMP1 in hfMSC migration, we examined whether the inhibition of PAR1 proteolysis may affect the ability of MSC to migrate towards HCC conditioned medium. The PAR1 proteins were first determined by western blot analysis. All of the representative fetal MSC isolates expressed similar levels of PAR1 proteins, including the representative highly migratory adult MSC-1 (Figure 3A). We then determined the effect of inhibiting PAR1 activation with anti-PAR1 monoclonal antibody (ATAP2), which specifically binds to the cleavage domain of PAR1, thus preventing the proteolysis of PAR1 by MMP1. The highly migratory MSC-1 was included as positive control. As shown in Figure 3Bi and Bii, anti-PAR1 treatment cause a significant reduction in the number of MSC (MSC-1, S8, S33) migrating to the conditioned medium derived from HCC in comparison to naïve cells and control IgG_1-treated cells. With the exception of S8, similar treatment with control IgG_1 did not cause a significant reduction in the number of migrating MSC. Taken together, the data suggested that there may be other cell factors that can bind and activate PAR1 receptor, which subsequently modulate the migration of hfMSC.

	Source	Migration	Percentage of cell migration normalized to MSC-1
MSC-19	Adult	High	**151**
MSC-13	Adult	High	**119**
MSC-1	Adult	High	**100**
MSC-7	Adult	High	**73**
MSC-15	Adult	High	**79**
MSC-18	Adult	High	**79**
MSC-3	Adult	High	**67**
MSC-16	Adult	High	**67**
MSC-17	Adult	High	**57**
MSC-5	Adult	High	**56**
MSC-10	Adult	High	**55**
MSC-2	Adult	Low	**11**
MSC-22	Adult	Low	**14**
MSC-6	Adult	Low	**15**
MSC-11	Adult	Low	**39**
MSC-23	Adult	Low	**45**
MSC-20	Adult	Low	**49**
MSC-21	Adult	Low	**49**
MSC-8	Adult	Low	**24**
MSC-9	Adult	Low	**46**
S-28	Fetal	High	**156**
S-8	Fetal	High	**144**
S-27	Fetal	High	**140**
S-38	Fetal	High	**114**
S-9	Fetal	High	**113**
S-43	Fetal	High	**90**
S-34	Fetal	High	**88**
S-64	Fetal	High	**83**
S-33	Fetal	High	**82**
S-36	Fetal	High	**63**

Table 1: Migratory activity of various MSC isolates.

In a recent article, MMP2 has been shown to mediate the tropism of human umbilical cord blood-derived stem cells in a human medulloblastoma tumor model [33]. Thus, we proceed to determine the endogenous expression of MMP2 by western blot analysis in the various isolates. The expression of total MMP2 was relatively similar in all hfMSC (Figure 4A). To further confirm the possible role of MMP2, an ELISA assay for MMP2 was performed with the conditioned media derived from MSC-1 and human fetal MSC isolates (Figure 4B). We observed a high level of secreted MMP2 from S27 although the migration activity was the lowest among the four hfMSC isolates. In contrast, S33 which exhibited similar migration activity to S27 has the lowest MMP2 level, suggesting that MMP2 does not play a key role in the tumor tropism of hfMSC.

The activation of Rho proteins is mediated by specific guanine-nucleotide exchange factors, one of which is identified as p115RhoGEF [34]. However, p115RhoGEF can also activate as well as inhibit Rho signaling after stimulation of PAR1 [35,36]. From our earlier study, we showed that PAR1 may be activated independent of the expression and activity of MMP1. Here, we ask if Gα12/13 heteromeric proteins and the downstream signaling cascade may be involved in transducing the signals from PAR1 to promote hfMSC migration. It has been shown that Gα13 can stimulate RhoGEF activity of p115RhoGEF and directly couple to RhoA activation [37]. Gα12 does not activate Rho through p115 [38]. In the hfMSC that exhibit the highest migratory potential, Gα13, p115RhoGEF, RhoA, Rac and cdc42 proteins were significantly enhanced when compared to S27, i.e. the hfMSC that migrate the least among the four representative isolates (Figure 5). The protein profiles of the Gα13/p115RhoGEF/RhoA pathway in S8 resembled those observed in the highly migratory adult MSC-1 whereas the S33 and S64 shared similar protein expression levels to S27 in many of the signaling mediators. The G12/G13 α subunits were consistently higher in S33 compared to S27 or S64. The enhanced expression of Gα13/

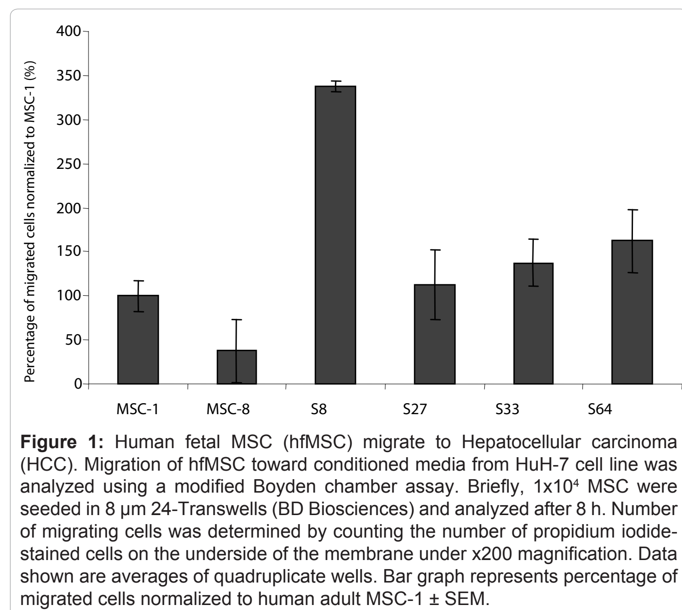

Figure 1: Human fetal MSC (hfMSC) migrate to Hepatocellular carcinoma (HCC). Migration of hfMSC toward conditioned media from HuH-7 cell line was analyzed using a modified Boyden chamber assay. Briefly, $1x10^4$ MSC were seeded in 8 µm 24-Transwells (BD Biosciences) and analyzed after 8 h. Number of migrating cells was determined by counting the number of propidium iodide-stained cells on the underside of the membrane under x200 magnification. Data shown are averages of quadruplicate wells. Bar graph represents percentage of migrated cells normalized to human adult MSC-1 ± SEM.

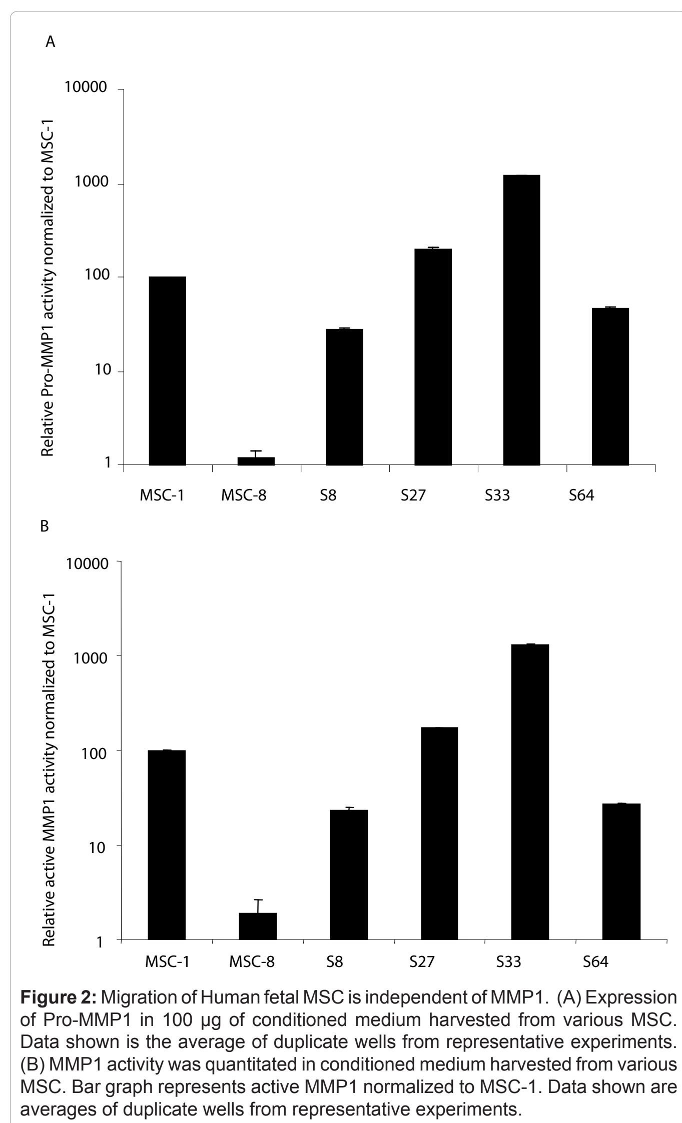

Figure 2: Migration of Human fetal MSC is independent of MMP1. (A) Expression of Pro-MMP1 in 100 µg of conditioned medium harvested from various MSC. Data shown is the average of duplicate wells from representative experiments. (B) MMP1 activity was quantitated in conditioned medium harvested from various MSC. Bar graph represents active MMP1 normalized to MSC-1. Data shown are averages of duplicate wells from representative experiments.

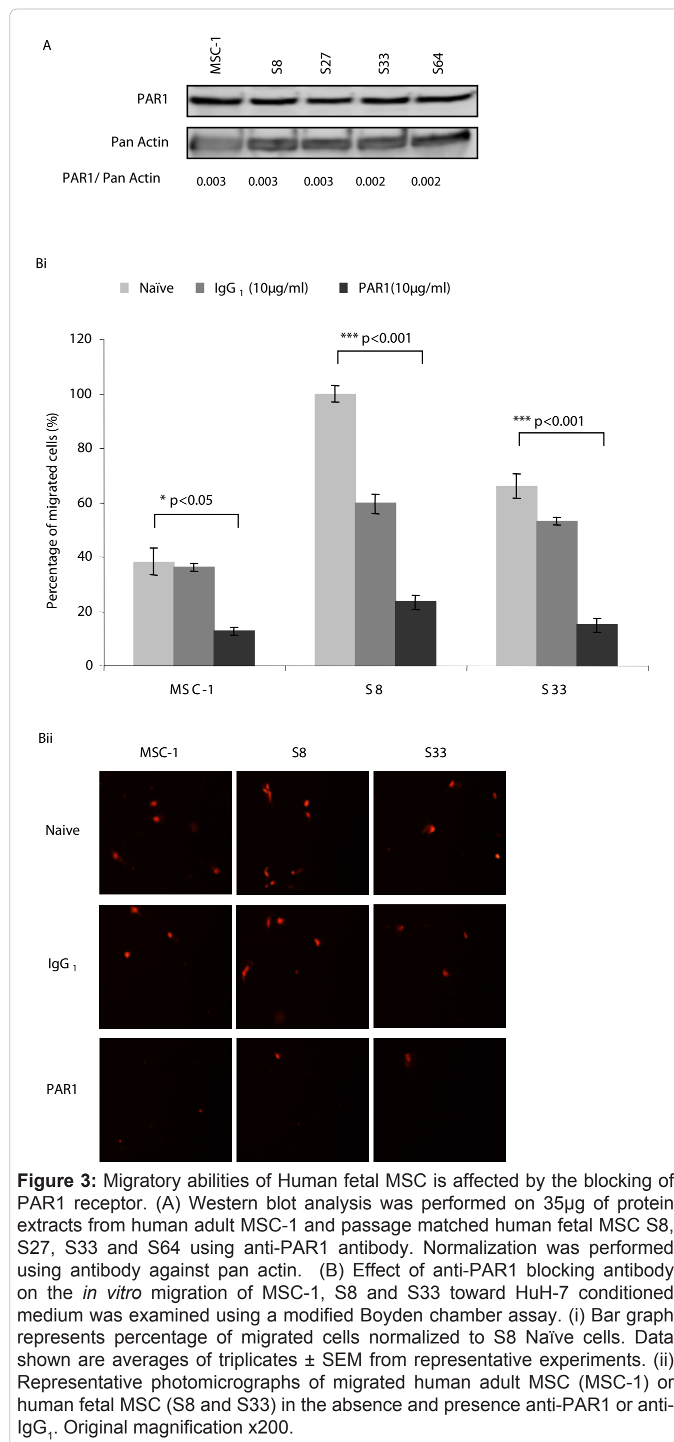

Figure 3: Migratory abilities of Human fetal MSC is affected by the blocking of PAR1 receptor. (A) Western blot analysis was performed on 35µg of protein extracts from human adult MSC-1 and passage matched human fetal MSC S8, S27, S33 and S64 using anti-PAR1 antibody. Normalization was performed using antibody against pan actin. (B) Effect of anti-PAR1 blocking antibody on the *in vitro* migration of MSC-1, S8 and S33 toward HuH-7 conditioned medium was examined using a modified Boyden chamber assay. (i) Bar graph represents percentage of migrated cells normalized to S8 Naïve cells. Data shown are averages of triplicates ± SEM from representative experiments. (ii) Representative photomicrographs of migrated human adult MSC (MSC-1) or human fetal MSC (S8 and S33) in the absence and presence anti-PAR1 or anti-IgG$_1$. Original magnification x200.

p115RhoGEF/RhoA proteins in highly migratory S8 suggests that this pathway could be involved in MSC migration although further analysis is required.

Discussion

In this study, we demonstrated that different isolates of hfMSC exhibited a more uniform and consistent tumor tropism when compared with adult human MSC isolates. In particular, we did not observe any hfMSC that failed to migrate towards conditioned medium

derived from tumor cells. Our results showed that the migration of hfMSC did not correlate to the expression and activity of MMP1 and MMP2. Rather, it appeared to be dependent, in part, on PAR1 expression, which may be modulated by GPCR signal pathways. Additional functional studies are required to confirm the precise underlying migratory mechanism.

Unlike the adult MSC, we did not observe a tight correlation between MMP1 and fetal MSC migration (Figure 1 and Figure 2). Likewise, significant levels of total and secreted MMP2 proteins could be detected using western blot and ELISA analysis in S27 when it migrate the least among the four representative hfMSC (Figure 4). However, when the MMP1 cognate receptor (PAR1) was blocked in the presence of anti-PAR1 antibodies (Figure 3Bi and ii), there was a significant reduction in the number of hfMSC migrated towards tumor-derived conditioned medium, suggesting that additional cellular factors other than MMPs may interact with PAR1 to modulate MSC migration. A likely candidate may be thrombin which is known to bind and subsequently cleaves the N-terminal domain of PAR1 which then acts as a tethered ligand to initiate intracellular signaling [39]. The activation of PAR1 resulted in the up-regulation of gene products involved in adhesion ($\alpha_{IIb}\beta3$, $\alpha v\beta5$, $\alpha v\beta3$ integrins) [40,41] invasion (MMP-2) [42], proinflammatory molecules (IL-6, macrophage inflammatory protein-1α) [43,44] and angiogenesis (IL-8; VEGF; bFGF; PDGF) [45-47]. In the adult MSC, thrombin can enhance MSC migration [45] although its role in the migratory activities of fetal MSC is currently unknown.

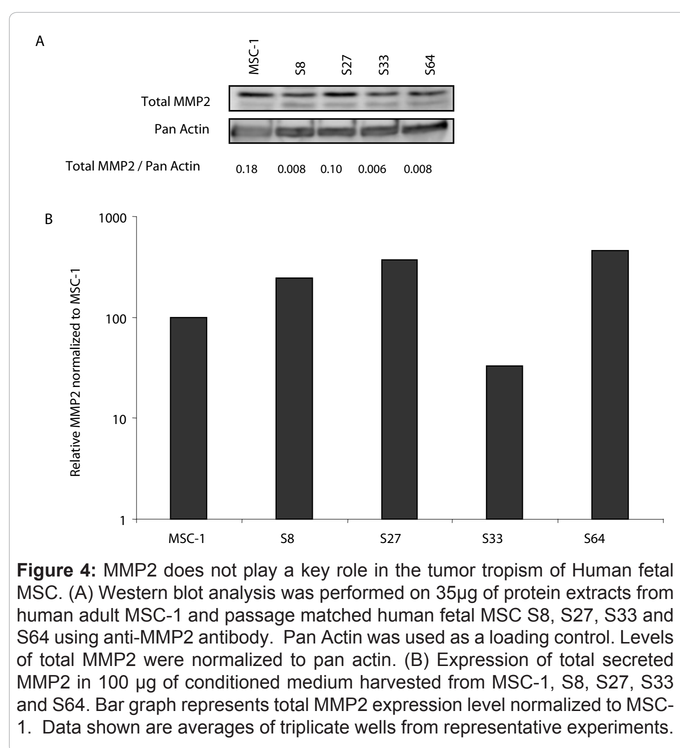

Figure 4: MMP2 does not play a key role in the tumor tropism of Human fetal MSC. (A) Western blot analysis was performed on 35µg of protein extracts from human adult MSC-1 and passage matched human fetal MSC S8, S27, S33 and S64 using anti-MMP2 antibody. Pan Actin was used as a loading control. Levels of total MMP2 were normalized to pan actin. (B) Expression of total secreted MMP2 in 100 µg of conditioned medium harvested from MSC-1, S8, S27, S33 and S64. Bar graph represents total MMP2 expression level normalized to MSC-1. Data shown are averages of triplicate wells from representative experiments.

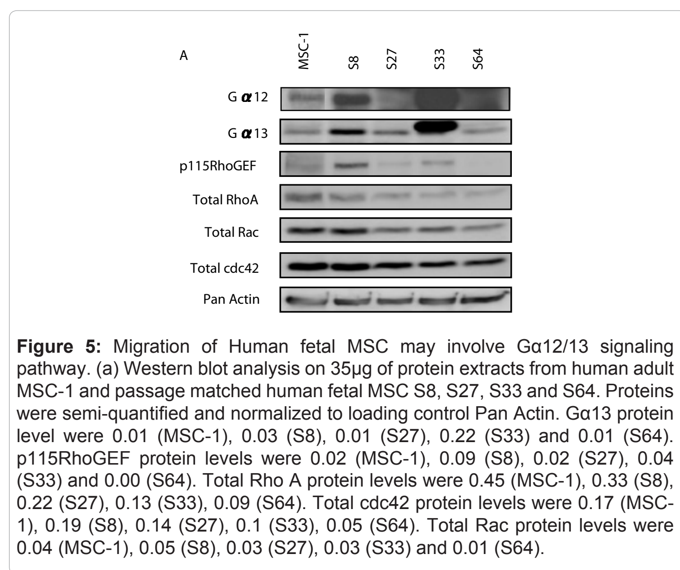

Figure 5: Migration of Human fetal MSC may involve Gα12/13 signaling pathway. (a) Western blot analysis on 35µg of protein extracts from human adult MSC-1 and passage matched human fetal MSC S8, S27, S33 and S64. Proteins were semi-quantified and normalized to loading control Pan Actin. Gα13 protein level were 0.01 (MSC-1), 0.03 (S8), 0.01 (S27), 0.22 (S33) and 0.01 (S64). p115RhoGEF protein levels were 0.02 (MSC-1), 0.09 (S8), 0.02 (S27), 0.04 (S33) and 0.00 (S64). Total Rho A protein levels were 0.45 (MSC-1), 0.33 (S8), 0.22 (S27), 0.13 (S33), 0.09 (S64). Total cdc42 protein levels were 0.17 (MSC-1), 0.19 (S8), 0.14 (S27), 0.1 (S33), 0.05 (S64). Total Rac protein levels were 0.04 (MSC-1), 0.05 (S8), 0.03 (S27), 0.03 (S33) and 0.01 (S64).

The Rho family of GTPases is key regulators of actin cytoskeletal dynamics and has been included in our studies to provide better understanding in regard to migration of hfMSC. Most RhoGTPases switch between an active GTP-bound form and an inactive GDP-bound form, which is modulated by three sets of proteins, namely the guanine nucleotide-exchange factors (GEFs), the GTPase-activating proteins (GAPs) and the guanine nucleotide-dissociation inhibitors (GDIs) [48,49]. In the context of Gα12/13 proteins, there are four RhoGEFs that are known to regulate the G proteins, namely the p115-RhoGEF, PSD-95/Disc-large/ZO-1 homology (PDZ)-RhoGEF, leukemia-associated RhoGEF (LARG) and the lymphoid blast crisis (Lbc)-RhoGEF. The binding of the RhoGEFs to Gα12/13 will stimulate its GEF activity [50,51]. Oligomerization of RhoGEF negatively regulates its activity, which in turn prevent interaction with RhoA [52,53]. RhoA which is involved in the formation of actin stress fibers and focal contact sites [54] is further regulated by GDIs [55].Other Rho family members such as Rac1 and Cdc42 which are also involved in cell migration are known to promote the formation of lamellipodia and filopodia respectively [56]. In our studies, S8 expressed slightly more Gα12/13 proteins in comparison to S27. The p115RhoGEF seemed to be expressed in an inverse correlation to RhoA. Further, the Gα13/p115RhoGEF/RhoA pathway is activated in S8 when compared to the other hfMSC. The protein profiles of the Gα13/p115RhoGEF/RhoA pathway in S8 resembled those observed in the highly migratory adult MSC-1. Coincidentally, S8 is derived from a more mature gestational age of 16 weeks when compared to S27 (week 13), S33 (week 10) and S64 (week 13). The possible role of Rho proteins in MSC migration has been inconsistent. The inhibition of Rho resulted in actin cytoskeleton rearrangement in MSC and rendered the cells susceptible to induction of migration by physiological stimuli [57]. By contrast, neither Rho nor Rho-effector is required for 3D migration of MSC [58]. In hematopoietic cells, Rho GTPases Rac1 and Rac2 certainly play distinct roles in actin organization and migration of hematopoietic progenitor cells, in addition to cell survival and proliferation [59]. In mouse myoblast C2C12 cells, bone-morphogenic protein-2 (BMP-2) -mediated cell migration has recently been shown to require both the activation of small GTPase Cdc42/PI3K and p38 pathways [60]. Taken together, our results showed that Gα13/p115RhoGEF/RhoA pathway may be involved in hfMSC although this requires further validation *in vitro* and *in vivo*.

Apart from Gα12/13 that could signal through Rho family member, other GPCR such as CXCR4 and its ligand SDF-1 also play an important role in regulating stem cell homing [61-63]. Although the endogenous status of SDF-1 and CXCR4 are not determined in this study, the SDF1/CXCR4 axis is well studied in human umbilical cord derived MSC (hUCB-MSC). In the latter cells, CXCR4 transcript

is reported as strongly expressed and functional in response to an SDF-1 gradient although the surface expression was low. In a recent study, SDF-1 was found to induce the migration of hUCB-MSC in a dose-dependent manner. The induced migration was subsequently inhibited by CXCR4-specific peptide antagonist (AMD-3100) and other inhibitors against PI3K, ERK and p38MAPK, suggesting that these signal transduction pathways may also be involved in SDF-1-mediated migration of hUCB-MSC [64]. Thus, the functional importance of CXCR4 may be in a dynamic cycles to and from the cell surface via endocytosis.

Taken together, unlike adult MSC, our results showed that the tumor tropism exhibited by hfMSC is not strictly correlated to the expression of MMPs levels. However, blocking the cognate receptor PAR1 resulted in significant reduction in MSC migration, suggesting that other additional factors may interact with PAR1 to modulate MSC migration. Alternately, other GPCR may also be involved in modulating the migratory activities of hfMSC. Nevertheless, our studies demonstrated that hfMSC exhibited uniformly high migratory activities to conditioned media derived from tumor cells, and could serve as good cellular vehicles for treatment of human cancers.

Acknowledgement

We would like to thank Dr. Alex Lyakhovich (Duke-NUS) for his valuable advice given to the Gα12/13 protein studies, Dr. IAW Ho (National Cancer Centre, Singapore) for her technical advice on the PAR1 inhibition study. We have used the same MSC-1 and MSC-8 as controls, which were mentioned from an earlier publication and would like to extend our appreciation once again to Dr. Guo Chang Ming (Singapore General Hospital) for providing the adult bone marrow. This research is supported by grants from the Singapore Stem Cells Consortium (A*STAR) and the National Medical Research Council Singapore. Dr. J Chan received salary support from the National Medical Research Council (NMRC/CSA/012/2009).

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This article was originally published in a special issue, **Stem Cell Based Therapy** handled by Editor(s). Dr. Aline M. Betancourt, Tulane University Medical Center, USA