Identification of chemokines and cytokines involved in the migration of MSC to bone marrow as well as to damaged tissues.

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Abstract

Background : Recent in vivo studies have demonstrated that transplanted MSC in mice were able to migrate into various tissues including lung, brain, bone marrow, heart and many others. Nevertheless, mechanisms responsible for the MSC migration remain unclear.

Methods : Bone marrow MSC (BM-MSC) were identified through their expression of SH2, SH3, CD44, CD105 but absence of expression of hematopoietic antigens: CD14, CD34, CD45 and CD62-E. The MSC migration, 5.10^5 cells/mL in the upper chamber, was evaluated using matrigel invasion chamber assay. In the bottom chamber, different cytokines were added for their chemoattractive properties : SDF-1, PDGF-bb, IL-6 (100ng/mL) and IGF (0,5 µg/mL) in a serum free medium. Blocking peptide or antibody against these cytokines were used to confirm the migration effect. BM-MSC conditionned medium (CM) at 10% was also used as chemoattractant. After 24 hours, cells that migrated through the membrane were counted.

Results : We observed that chemokines involved in the regulation of immunity and inflammation process promotes the migration of MSC. High migration is observed when 100ng/mL of PDGF-bb was used in the lower chamber. Through FACS analysis and semi-quantitative RT-PCR we noted that the migratory response to PDGF-bb, IL-6 and IGF is in relation with the high expression of their respective receptors. We also observed that MSC does not respond to SDF-1 since only very low level of extracellular expression of CXCR4 was observed. Our study, thus indicate that these chemokines could be involved in the migration of MSC to bone marrow as well as to damaged tissues.

Methods

- Bone marrow from sternal aspiration were seeded in alpha-minimal essential medium and MSC were isolated using the plastic adhesion method. Non adherent cells were removed after 24-48 hours of culture. When 70-80% of confluency was attempted, cells were detached with trypsin solution and reseeded (passage). After the second passage the phenotype, the plasticity and the migration potential were evaluated.

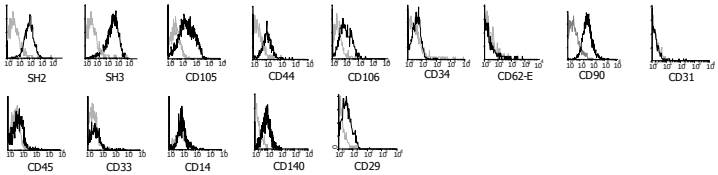
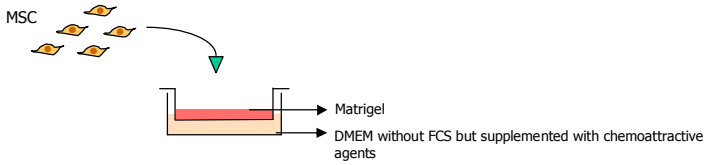


Figure 1: Phenotype profiling of MSC after two passages

- Migration assay:

Invasion chambers were prepared by coating inserts (8µm, Costar) with 50µL Matrigel.
MSC (5.10^5 cells/mL) were incubated for the migration assay during 24 hours in the upper chamber.



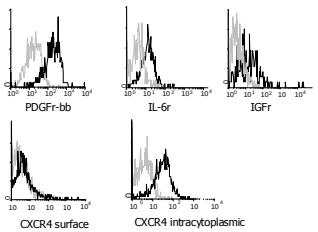
Results

Table1: Migration assay

	Ctrl	SDF-1	IGF	10%CM	IL-6	PDGFbb
Migrated MSC	13,7 ± 2,7	24,9 ± 5	42,2 ± 12,7	69 ± 17,9	77,1 ± 14,1	90,5 ± 4,3

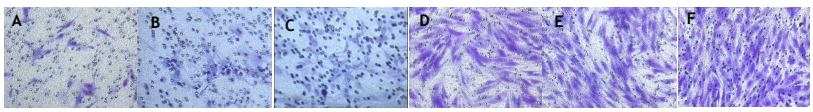
After the second passage, the migratory property of MSC was evaluated using Boyden Chamber coated with Matrigel. Cells (5.10^5 cells/mL) were seeded in the upper chamber. The MSC migration was tested with several chemoattractive agents: SDF-1, IGF, Conditioned medium (CM), IL-6 and PDGF-bb. After 24 hours, migrated MSC were counted in three different fields. Data represent mean±SEM of 14 experiments of MSC through matrigel chambers.

Figure 2: Chemokine receptor expression



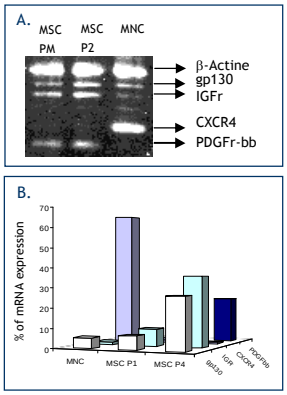
Through FACS analysis we demonstrated that MSC express PDGFr-BB, IL-6r (CD130, the signal transducing protein), IGFr and at a low level CXCR4 on their surface. We observed high level of intracytoplasmic CXCR4.

Figure 3: Migration assay



On the upper chamber, treated with martigel, 5.10^5 cells/mL were seeded and incubated during 24 hours. Chemoattractive properties of different agents in the lower chamber were evaluated : SDF-1 (B;100ng/mL), IGF (C; 0,5 µg/mL), IL-6 (E; 100ng/mL) and PDGF-bb (F; 100ng/mL in a serum free medium. BM-MSC conditioned medium (CM) at 10% was also used as chemoattractant(D). The spontaneous migration of MSC was evaluated using medium without FCS (A). After 24 hours, cells were colored with crystal violet solution (0,5%) and three different fields were counted.

Figure 4: RT-PCR analysis



Through multiplex PCR analysis (A), we confirmed the expression of cytokine receptors implicated in the migratory potential of MSC. We observed that mononuclear cells (MNC) have no expression of PDGFr-bb and only a weakly expression of IGFr. Single RT-PCR using MNC and different samples of MSC revealed a slight CXCR4 expression that was not observed in the multiplex PCR (data not shown). In contrast, CXCR4 is highly expressed on MNC and decreased after MSC expansion.

Semi-quantitative PCR using ß-actin as an internal control. The results are expressed as percentage of ratio « chemokine receptors »/ ß-actin (B).

Discussion-conclusions

Recent in vivo studies have demonstrated that transplanted MSC in mice were able to migrate into various tissues including lung, brain, bone marrow, heart and many others. Nevertheless, mechanisms reponsible of the migration of MSC remain unclear. In this study, we evaluated the effect of some chemokines and cytokines(PDGF-bb, Il-6, IGF-I or BM-MSC conditioned medium) in the MSC trafficking using boyden chamber. The chemoattractive properties of these cytokines were compared to the spontaneous migration in presence of DMEM medium without FCS. We observed that chemokines involved in the regulation of immunity and inflammation process promotes the migration of MSC. The best migration is observed when 100ng/mL of PDGF-bb was used in the lower chamber as attractive chemokines. Through FACS analysis and semi-quantitative RT-PCR we noted that the migratory response to PDGF-bb, IL-6 and IGF is due to the highly expression of their respective receptors. We aslo demonstrated that MSC are not sensitive to SDF-1 since only very low level of extracellular expression of CXCR4 was observed.

In our study, we thus demonstrated that these chemokines could be involved in the migration of MSC to bone marrow as well as to damaged tissues.