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Brief communication (Original)

TNF-α and G-CSF induce CD62L and CD106 expressions on rat bone marrow-derived MSCs

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Background: Accumulating evidence suggests that CD62L and CD106 are positively expressed on the surface of mesenchymal stem cells (MSCs). It has been reported that both receptors can be induced by tumor necrosis factor-□ (TNF-α), granulocyte-colony stimulating factor (G-CSF), and vascular endothelial growth factor (VEGF) on leucocytes. However, whether these stimulations induce CD62L and CD106 expressions on MSCs is still unknown. Thus, in the present study we investigated the effects of TNF-α, G-CSF and VEGF on CD62L and CD160 expressions on the surface of MSCs.
Method: MSCs were isolated from rat bone marrows, and treated with different concentrations of TNF- α (0.1, 1 and 10 ng/mL), G-CSF and VEGF (1, 10, and 100 ng/mL) for 12 and 24 hours respectively. Then the expressions of CD62L and CD106 on the surface of MSCs were analyzed by flow cytometry.
Results: Immunochemistry assay showed positive CD90 but negative CD45 in the MSCs. Flow cytometry analysis suggested that TNF-α and G-CSF could induce CD62L and CD106 expressions on the surface of MSCs in a dose-dependent manner, but not in a time-dependent manner. Further, all the concentrations of VEGF had no significant effect on the CD62L and CD106 expressions.
Conclusion: CD62L and CD106 can be induced by TNF-α and G-CSF on the surface of MSCs, but not by VEGF. These findings can help improve BM-MSC migration capability and therapeutic efficiencies of MSC transplantation.

Keywords: CD106, CD62L, G-CSF, mesenchymal stem cells, TNF-α

Mesenchymal stem cells (MSCs) are a kind of multipotentient progenitor cells that are derived from several kinds of tissues including bone marrow, umbilical cord, umbilical cord blood, synovial tissues, adipose tissues, and a number of fetal tissues [1]. These cells can differentiate into numerous cell types, including osteoblasts, chrondrocytes, hepatocytes, myocytes and adipocytes, and can expand rapidly *in vitro* or in vivo without discernible chromosomal abnormalities [1, 2].

In past decades, MSCs have been explored as an alternative source for cell transplantation and therapy in clinic. Currently, their clinical uses appear very promising. The preclinical and clinical studies have provided evidence indicating that MSC transplantations were beneficial in the treatment of a variety of diseases, such as myocardial infarction, cerebral infarction, stroke, osteoarthritis, hepatic failure, and the complications of diabetes mellitus [3-6].

MSC therapy has limitations due to the poor viability after cell transplantation. Selectin and integrin-mediated cell adhesion is a prerequisite for cell survival [7]. Cell-cell adhesion of MSCs is also essential for the MSC-dependent tissue regeneration after transplantations [8]. It has been reported that upregulation of adhesive molecules on the surface of MSCs facilitates their survivals [7, 9]. CD62L, which belongs to the L-selectin family and CD106, which is known as vascular cell adhesion molecule-1 (VCAM-1), are important adhesive molecules and have been detected to be positively expressed on the surface of MSCs [8].

Recent evidence suggests that CD62L and CD106 can be induced by tumor necrosis factor-α (TNF-α), granulocyte-colony stimulating factor (G-CSF), and vascular endothelial growth factor (VEGF) on the in vitro cultured leucocytes [10]. However, currently, whether stimulatory factors induce CD62L and CD160 on the surface of MSCs is still not known.

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Therefore, the purpose of the present study was to investigate the effects of TNF-α, G-CSF, and VEGF on the expressions of CD62L and CD106s on the surface of rat bone morrow-derived MSCs (BM-MSCs).

Materials and methods

Experimental animals

Three-month-old Sprague Dawley male rats, weighing 500□20g were provided by Experimental Animal Center of Luzhou Medical College (Luzhou, China). All the experiments were conducted in accordance with the national guidelines for the care and use of laboratory animals. This study was approved by the Ethnic Committee of The Affiliated Hospital, Luzhou Medical College (Luzhou, China).

Cell culture

MSCs were isolated from rat bone marrow and cultured in Low Carbohydrates Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) in an atmosphere of 5% CO_2 at 37°C. After 48 hours, the non-adherent cells were removed by changing the media. The media were replaced every three days. The 3-passage MSCs were used in the following experiments. Cell morphology was observed using an inverted phase contrast microscope (Olympus, Inc., Tokyo, Japan) every day.

Pretreatment with TNF-□, G-CSF, and VEGF

Cells were seeded into 6-well plates (2×10^5 / well) and treated with various concentrations of TNF-α (0.1, 1, and 10 ng/mL), G-CSF and VEGF (1, 10, and 100 ng/mL) for 12 or 24 hours. The untreated cells served as blank controls.

Immunochemistry analysis

For immunochemistry assay, BM-MSCs were seeded and cultured on the fibronectin pretreated coverslips in 24-well plates. After washing for three times, the cells were fixed in 4% paraformaldehyde for 30 minutes, and then treated with 90% methanol/ 3% H_2O_2 for 15 minutes at room temperature. As following, the cells were blocked by 10% goat serum for 30 minutes at room temperature, and subsequently co-incubated with anti-CD45 and anti-CD90 antibodies (BioLegend, San Diego, CA, USA) (1:500) respectively and overnight at 4°C. The cells were washed for three times and incubated in biotin-conjugated second antibodies (BioLegend, San Diego, CA, USA) for 30 minutes. The cells were incubated with SABC solution for 20 minutes. The washed coverslips were then treated with DAB solution and the reaction was suspended after 10 minutes.

Flow cytometry analysis

For flow cytometry (FACS) analysis, cells (2×10^5/well) were digested and collected after the stimulatory treatments. The collected cells were washed for three times and treated with 1% BSA for 10 minutes, and then incubated with FITC-conjugated CD62L antibody and phycoerythrin (PE)-conjugated CD 106 antibody (BioLegend, San Diego, CA, USA) for 30 minutes in the dark. After washing for three times, the samples were analyzed by a flow cytometry (Epics XL, Beckman Coulter, Inc., Brea, CA, USA).

Statistical analysis

All calculations were performed with SPSS 12.0 software (SPSS Inc., Chicago, IL, USA). Numerical data were presented as means□standard deviations. One-way ANOVA were used to compare the differences among groups. A *P* value of less 0.05 was considered significant.

Results

BM-MSCs identification

When the cells reached a confluence of 75-85%, the cellular morphology was imaged with an inverted phase contrast microscope. The result showed that MSCs exhibited fibroblast-like morphology, and intensively arrayed in a vortex-like pattern. The specific markers were characterized by immunochemistry staining. It was found that MSCs were positive for CD90, but negative for CD45 (**Figure 1**).

Expressions of CD62L and CD106

TNF-α and G-CSF could increase expressions of CD62L and CD106 on the surface of BM-MSCs, which appears a significant dose-response (CD62L: TNF-α: control vs. 100 pg, 100 pg vs 1 ng and 1 ng vs. 10 ng, $p < 0.01$; G-CSF: control vs. 1 ng, 1 ng vs. 10 ng and 10ng vs. 100ng, $p < 0.01$) (CD106: TNF-α: control vs. 100 pg, $p > 0.05$; 100 pg vs. 1 ng, $p < 0.05$; 1 ng vs. 100ng, $p < 0.01$; G-CSF: control vs. 1 ng, 1 ng vs. 10 ng and 10 ng vs. 100 ng, $p > 0.05$; 1ng vs. 100 ng, $p < 0.05$; G-CSF: control vs. 100 pg, $p > 0.05$; 100 pg vs. 1 ng, $p < 0.05$; 1ng vs. 100 ng, $p < 0.01$; G-CSF:

control vs. 1 ng, 1 ng vs. 10 ng and 10 ng vs. 100 ng, $p > 0.05$; 1 ng vs. 100 ng, $p < 0.05$), but has no the time-response (12 hours vs. 24 hours, $p > 0.05$). Treatments with VEGF (any concentrations) did not affect the expressions of CD62L and CD106 on the surface of BM-MSCs as shown in **Figures 2 and 3**.

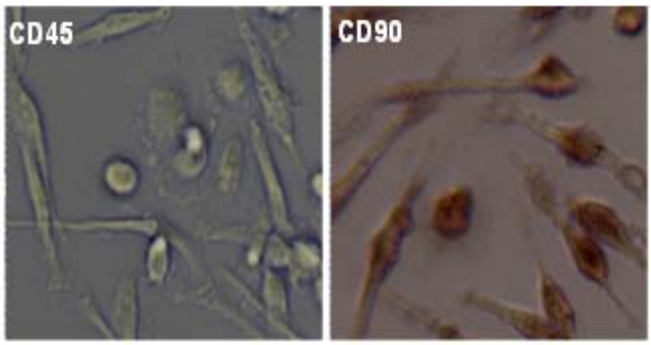

Figure 1. Immunochemistry shows that MSCs were positive for CD90, but negative for CD45 (200×)

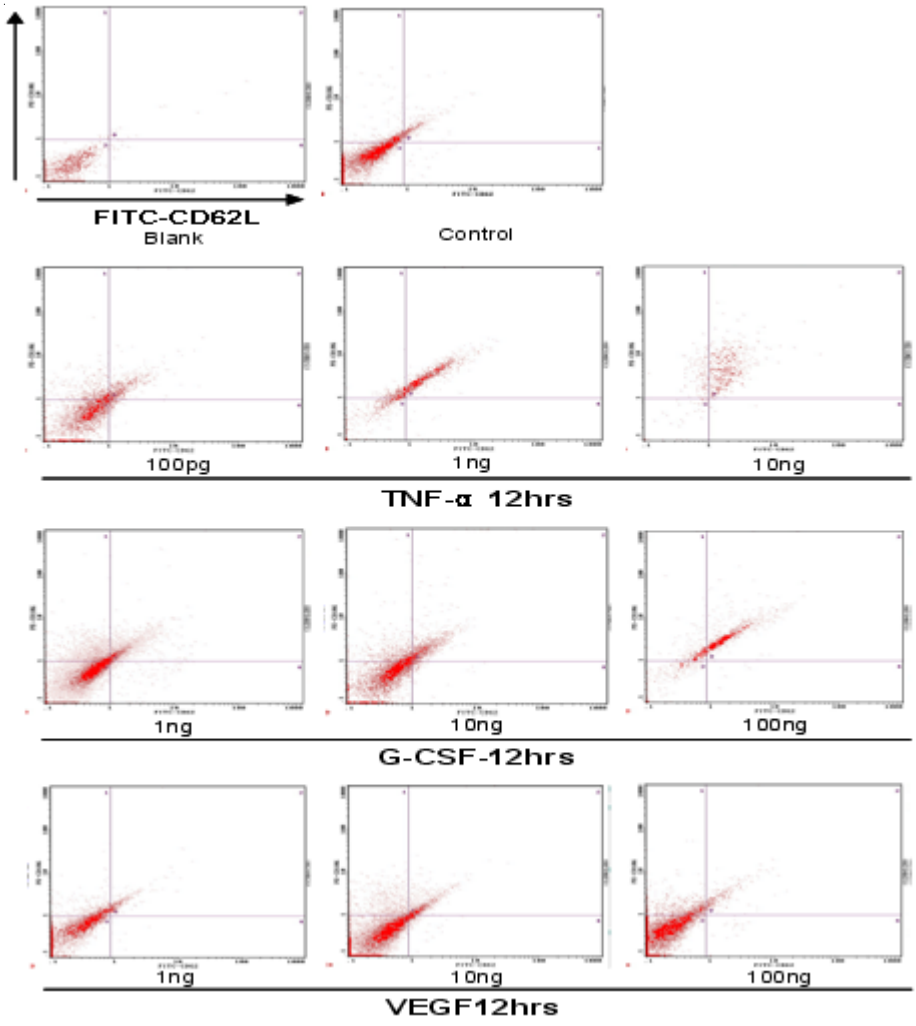

Figure 2. Flow-cytometry pictures shows that the effect of different concentrations of TNF-α, G-CSF and VEGE on CD62L (FITC-conjugated) and CD106 (PE-conjugated) expressions on the surface of MSCs after 12 hours treatment.

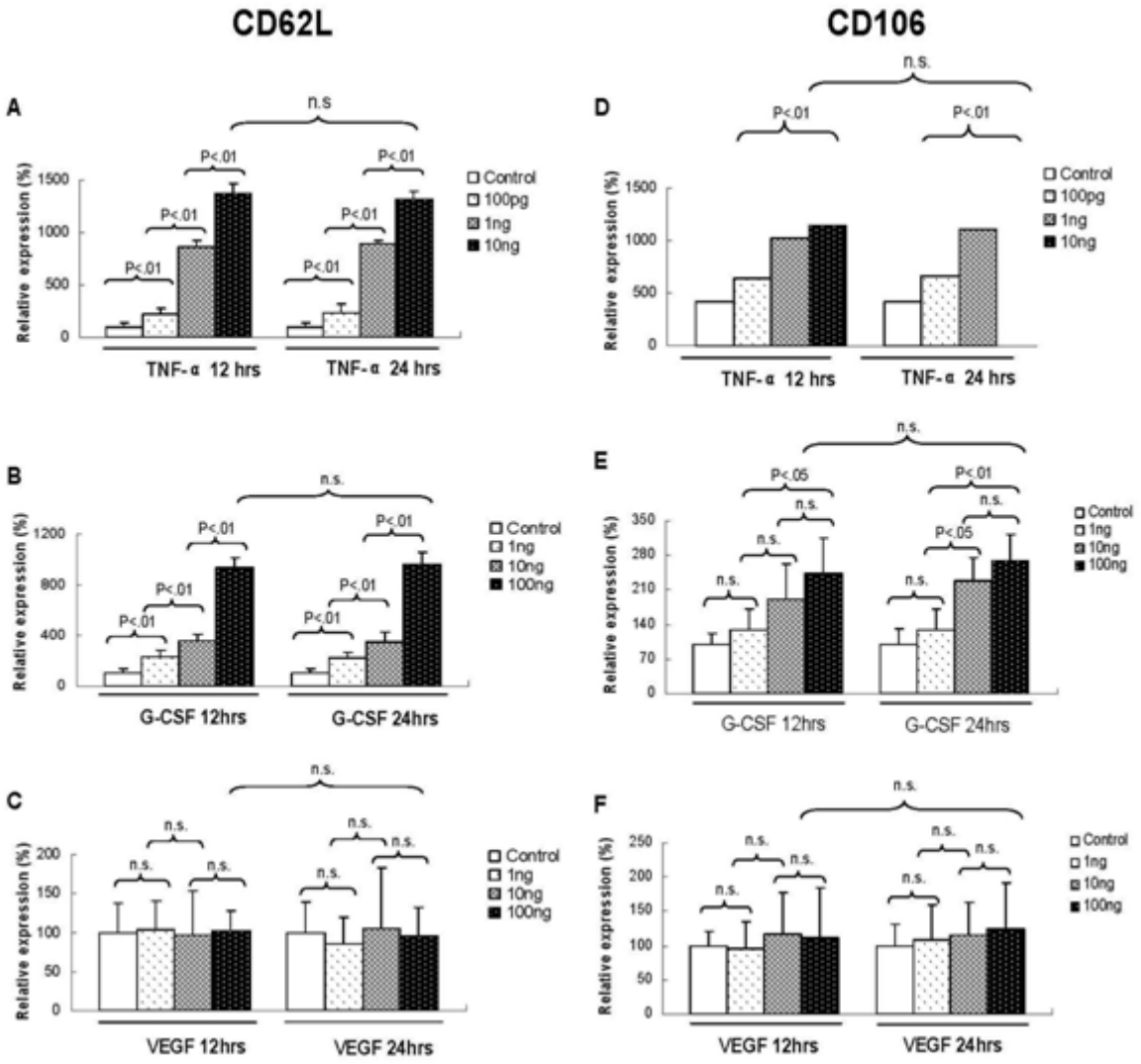

Figure 3. Flow-cytometry analysis shows that the results CD62L and CD106 expressions on the surface of MSCs treated with different concentrations of TNF-α, G-CSF, and VEGE at 12 and 24 hours. Bar graphs represent mean±SD (n = 4 per group).

Discussion

BM-MSCs are a subset of non-hematopoietic stem cells, characterized by their ability of self-renewal and differentiation into multiple cell types, including neuron-like cells, osteoblasts, chrondrocytes, adipocytes, myocytes, and adipocytes [2, 16]. BM-MSCs are described to be positive for CD44, CD90, CD29, CD73, CD105, and CD166 and negative for CD14, CD34, and CD45 [11]. Under the in vitro cultured conditions, their morphology is the typical fibroblast. Consistent with the previous data from other groups, our results showed that BM-MSCs exhibited a fibroblast-like morphology, and arrayed in a vortex-like pattern in the cultured states and were positive for CD90, but negative for CD45.

Due to MSCs with high reproducible characteristics and multipotentient differentiation ability, they have been viewed as excellent candidates for cell therapy [2, 12]. BM-MSC transplantation and therapy have offered a great promise in would healing and bone repair tissue engineering. It has been demonstrated that transplantation of BM-MSCs into the ischemic brain or the post-MI heart would enhance the performances of the injured brains or hearts [13, 14]. Although MSC therapy seems promising, it is also confronted with multiple fundamental problems. In the current, the most basic of these problems is how to improve the migration rate of the transplanted MSCs from the injection site to the injured regions [15]. There is much evidence that BM-MSCs have certain abilities

to migrate into and repair the injured tissues and organs after various infusions, such as intravenous, intra-arterial and intrathecal injections [16, 17]. Previous studies have shown that MSCs can circulate with blood or lymphyatic flow, migrate in the peripheral tissues, eventually home into different organs. However, it has been also demonstrated that the organ-homing rate of MSCs after injections is very low [17]. Currently, many strategies have been developed to improve the migratory and homing capacity of MSCs. Among these strategies, induction culture in vitro, especially exposed to the cytokines, is the most commonly used one.

Cell adhesion is the prerequisite for MSC migration from circulation system into peripheral tissues. Cell migration from circulation system to peripheral tissues is an adhesive and transendothelial migration process. The expressions of chemokine receptors and adhesion molecules play a crucial role in this process. Moreover, local transplantation is another important MSC administration technique, which has been adopted by many researchers. Adhesion of MSCs delivered by this method is also required for their localizations in the injection regions [18]. The most important adhesive receptors that determine BM-MSC migratory abilities are selectins, integrins, VCAM-1 and ICAM-1. Previous studies have showed that up-regulation of these adhesive molecules facilitates BM-MSC migration, improves their survivals and enhances their therapeutic potentials [7, 9].

CD62L, also known as L-selectin and LECAM-1, is constitutively expressed on all classes of leukocytes. CD106 (VCAM-1), the receptor for VLA-4, is another important cell surface protein generally expressed on the activated endothelial cells or certain classes of leukocytes**.** Both receptors are important mediators of leukocyte adhesion to blood vessel walls. The related studies suggest that MSC adhesion and migration in blood vessels and peripheral tissues are similar with leukocytes [19]. CD62L and CD106 have been found positively expressed on the surface of MSCs, and their expression levels directly affect the organ homing and survival rates of BM-MSCs after intravenous or intra-arterial injections [8]. In this study, we exposed BM-MSCs to different stimulatory factors (TNF-α, G-CSF and VEGF) and tested the expressions of CD62L and CD106 on the surface of BM-MSCs. Our results showed that TNF-α and G-CSF up-regulated the expressions of two receptors in a dose response manner. However, VEGF had no effect on their expressions. This result showed that in vitro culture with the induction of TNF-α and G-CSF may be a useful strategy to facilitate MSC migration and enhance the therapeutic potentials of M-MSC transplantation in clinic.

In conclusion, our findings showed that TNF-α and G-CSF increased the expressions of CD62L and CD106 on the surface BM-MSCs in a dose response manner, but not VEGF. These findings may provide a piece of valuable information to improve BM-MSC migration capability and better therapeutic efficiencies of MSC transplantation engineering.

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References

1. Bolan-Meade J, Vogelsang GB. Mesenchymal stem cells and organ transplantation: current status and promising future. Transplantation. 2006; 81:1288-389.
2. Jones E, McGonagle D. Human bone marrow mesenchymal stem cells in vivo. Rheumatology. 2007; 11:1-6.
3. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, et al. Bone marrow cells regenerate infracted myocardium. Nature. 2001; 410:701-5.
4. Davatchi F, Abdollahi BS, Mohyeddin M, Shahram F, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. Int J Rheum Dis. 2011; 14:211-5.
5. Volarevic V, Arsenijevic N, Lukic ML. Concise review: Mesenchymal stem cell treatment of the complications of diabetes mellitus. Stem Cells. 2011; 29:5-10.
6. Bao X, Wei J, Feng M, Lu S, Li G, Dou W, et al. Transplantation of human bone marrow-derived mesenchymal stem cells promotes behavioral recovery and endogenous neurogenesis after cerebral ischemia in rats. Brain Res. 2011; 1367:103-13.
7. Song SW, Chang W, Song BW, Song H, Lim S, Kim HJ, et al. Integrin-linked kinase is required in hypoxic mesenchymal stem cells for strengthening cell adhesion to ischemic myocardium. Stem cells. 2009; 27:1358-65.
8. Nishihira S, Okubo N, Takahashi N, Ishisaki A, Sugiyama Y, Chosa N. High-cell density-induced VCAM1 expression inhibits the migratory ability of

mesenchymal stem cells. Cell Biol Int. 2011; 35:475-81.
9. Zhang D, Fan GC, Zhou X, Zhao T, Pasha Z, Xu M, et al. Over-expression of CXCR4 on mesenchymal stem cells augments myoangiogenesis in the infarcted myocardium. J Mol Cell Cardiol. 2008; 44: 281-92.
10. Grailer JJ, Kodera M, Steeber DA. L-selectin: Role in regulating homeostasis and cutaneous inflammation. J Dermatol Sci. 2009; 56:141-7.
11. Jones EA, English A, Kinsey SE, Straszynski L, Emery P, Ponchel F, et al. Optimization of a flow cytometry-based protocol for detection and phenotypic characterization of multipotent mesenchymal stromal cells from human bone marrow. Cytometry B Clin Cytom. 2006; 70:391-9.
12. Pittenger MF, Martin BJ. Mesenchymal stem cells and their potential as cardiac therapeutics. Circ Res. 2004; 95:9-20.
13. Toyama K, Honmou O, Harada K, Suzuki J, Houkin K, Hamada H, et al. Therapeutic benefits of angiogenetic gene-modified human mesenchymal stem cells after cerebral ischemia. Exp Neurol. 2009; 16:47-55.
14. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med. 2006; 355:1199-209.
15. Johnson TV, Bull ND, Martin KR. Identification of barriers to retinal engraftment of transplanted stem cells. Invest Ophthalmol Vis Sci. 2010; 51:960-70.
16. Lundberg J, Le Blanc K, Soderman M, Andersson T, Holmin S. Endovascular transplantation of stem cells to the injured rat CNS. Neuroradiology. 2009; 51:661-7.
17. Kan I, Melamed E, Offen D. Integral therapeutic potential of bone marrow mesenchymal stem cells. Curr Drug Targets. 2005; 6:31-41.
18. Herrmann JL, Abarbanell AM, Weil BR, Wang Y, Poynter JA, Manukyan MC, et al. Postinfarct intramyocardial injection of mesenchymal stem cells pretreated with TGF-alpha improves acute myocardial function. Am J Physiol Regul Integr Comp Physiol. 2010; 299: R371-R378.
19. De Becker A, Van Hummelen P, Bakkus M, Vande Broek I, De Wever J, De Waele M, et al. Migration of culture-expanded human mesenchymal stem cells through bone marrow endothelium is regulated by matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-3. Haematologica. 2007; 92:440-9.