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Research Article

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The effect of Rho/ROCK signal pathway on the migration of mesenchymal stem cells induced by IL-1β

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ABSTRACT

Mesenchymal stem cells can not only differentiate into multiple cells, but also targetly migrate to the sites of injury, chronic inflammation, and tumor, repairing these parts in a certain mechanism. Having a comprehensive knowledge of migration mechanism of mesenchymal stem cells and improving the "homing" index are helpful for autologous repair and cell therapy. It is reported that inflammatory cytokines IL-1β can induce the migration of mesenchymal stem cells, but the details of migration mechanism remains unclear. In this study, migration models of MSCs was established by treatment with 50ng/mL IL-1β. After 72 hours, wound heal assay and transwell assay showed that IL-1β could induce the migration of mesenchymal stem cells. Then, migration markers MYL9 and CYR61 were tested by RT-PCR and Western Blot. The results showed that the expression of both MYL9 and CYR61 were increased. Rho signal pathway is widely involved in cell migration. To determine whether Rho signaling pathway participated in the migration of mesenchymal stem cells induce by IL-1β, mesenchymal stem cells were pretreated with 10μmol/L the inhibitor of Rho signaling pathway-Y27632 for 0.5 hour before treatment with IL-1β. Wound heal assay and transwell assay both showed that Y27632 could reduced the migration of mesenchymal stem cells caused by IL-1β. RT-PCR and Western Blot showed that Y27632 down regulated the expression of MYL9 and CYR61 in both mRNA levels and protein levels.

Key words: mesenchymal stem cells, IL-1β, Rho, migration, inhibitor

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INTRODUCTION

Mesenchymal stem cells (MSCs)are a population of multipotential cells defined by their self-renewal capabilities[1-3].They are good candidates for cell therapy because of their differential potential, limited tendency to produce tumors, ease of isolation, immunologically privileged nature, and ability to home to damaged tissue[4-8].

Mesenchymal stem cells homing is a multi-factor, multi-step process in vivo. Many cytokines, chemokines, adhesion molecules and matrix metalloproteinases were involved in mesenchymal stem cell homing movement[9,10].Furthermore, mesenchymal stem cellshoming in vivo and migration in vitro, like other physiological activity of cells, were induced by different factors and different signaling pathways. Although a variety of factors and signaling pathways were found involved in the migration process of mesenchymal stem cells, but the molecular mechanisms is still unclear.

Interleukin is a very important family of cytokine, has more than a dozen members of this family currently. They play important roles in a series of physiological processes of immune cells such as mature, activation, proliferation and immune regulation[11,12]. IL-1β is one of interleukin family. Carrero et al found that IL-1β may upregulate the expression of the protein CLDNs, promote the migration of mesenchymal stem cells , and activate NF-κB signaling pathway[13].

Rho GTPase control dynamics of the cytoskeleton through many different effector proteins to regulate actin assembly structure, activity, and flip, then control adhesion, morphology and activity of the cells. In different cells, Rho activation is different for cell migration.

In this study, we investigated IL-1β induce-migration of mesenchymal stem cells in vitro, and the role of Rho signaling pathway in MSC migration induced byIL-1β.

EXPERIMENTAL SECTION

2.1 Cell culture. Rat bone marrow-derived MSCs (rBM-MSCs) were isolated from the femurs and tibias of male Sprague–Dawley rats (weight 90-100g) as described previously. Briefly, bone marrow mononuclear cells were obtained by Percoll (1.073g/mL) density gradient centrifugation. The cells were seeded in Dulbecco's modified Eagle's medium–low glucose (DMEM-LG, Hyclone) supplemented with 10% fetal bovine serum FBS at 37℃in humified air with 5% CO_2.rBM-MSCs were phenotypically characterized by the method published by Wang et al[14].

2.2 Scratch wound-healing assay. Cells were seeded in 24-well plates, incubated with IL-1β to a final concentration of 50ng/mL, or added Y27632 half an hour earlier before incubation if need an inhibitor, and then scratched with the narrow end of a sterile pipet tip. The remaining cells were washed twice with warm culture medium to remove cell debris. The width of the scratch was photographed using an inverted phase contrast microscope (Olympus, Tokyo, Japan) and measured using Hprsnap6 software after 72 hours. After using the software Image J count, index to migrate to a control group, according to the formula: Experimental group migration index = (the number of migrated cells in experimental group/the total number of cells in experimental group)/(the number of migrated cells in control group/the total number of cells in control group) made histogram.

2.3 Transwell chamber assay. Cells were harvested by trypsinization and then seeded into the upper chamber of the Transwell cell culture insert at 5×10^5cellsin 200μL of medium, or added Y27632 half an hour earlier before incubation if need an inhibitor. The lower chamber was filled with 600μLof medium containing IL-1β to a final concentration of 50ng/mL. Twenty-four hours later, cells in the upper chamber were removed using a cotton swab, and cells migrated to the lower side of the membrane were fixed with 4% paraformaldehyde and stained with Giemsa. The number of migrated cells was counted and photographed in five fields (the upper, the lower, the left, the right, and the middle) of three independent experiments.

2.4 RNA Extraction, cDNA Synthesis, and PCR Reaction. Total RNA was extracted from cells with TRIzol reagent (Invitrogen). After that, the cDNA synthesis was performed using the reverse transcription with random primers. The thermal cycle profile was as follows: denaturation for 30s at 95℃, annealing for 30s at 54-57℃ depending on the primers used, and extension for 30s at 72℃. PCR products were visualized on 2% agarose gels stained with ethidium bromide under ultraviolet (UV) transillumination. The primers used in this study are summarized in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control to show equal loading of the cDNA samples.

Table 1. Primers used in the present study

Gene	Primer sequence
GAPDH	F: 5'-ATTCAACGGCACAGTCAAGG-3' R: 5'-GCAGAAGGGGCGGAGATGA-3'
CYR61	F: 5'- GAAGAAATACCGGCCCAAAT-3' R: 5'- CAGACTGTAGAGGCGAAACGAC-3'
MYL9	F: 5'- AGGACCTGGAGGGTATGAT-3' R: 5'- CTTGAGGATGCGAGTGAAC-3'

2.5 Western Blot. The total protein of cells was prepared using extraction buffer composed of phosphate buffered saline (PBS) containing 0.5% Triton X-100, 1 mM EDTA, 1 mM phenylmethylsulfonylfluoride (PMSF) and protease inhibitors (Roche, Basel, Switzerland). Equal amount of total protein was loaded on 12% sodium dodecylsulfate polyacrylamide gel. Then samples were transferred to nitrocellulose membranes and blocked for 60 min at room temperature in 5% skim milk powder (wt/vol). The membranes were immunoblotted with anti-CCN1(1:500, Abcam, Cambridge, England), anti-MYL9(1:1000, Abcam, Cambridge, England)antibodies overnight at 4℃, and then incubated with IRDye™-800 conjugated anti-rabbit or anti-mouse secondary antibodies (Li-COR Biosciences, Lincoln, USA) for 30 min at room temperature. The specific proteins were visualized by Odyssey™ Infrared Imaging System (Gene Company, Hong Kong, China).β-actin was used as an internal control to show equal loading of the protein samples.

2.6 Statistical analysis. The data from the above mentioned quantification assays were expressed as mean ± SD. The statistical significance of differences was determined using Student's t test. The minimal level of significance was P< 0.05

RESULTS AND DISCUSSION

3.1IL-1β-induced mesenchymal stem cell migration. In order to study whether IL-1β can induce the migration of bone marrow mesenchymal stem cells, scratch repair ability of mesenchymal stem cells were observed by inverted phase contrast microscope after treatment with 50ng/mL IL-1β for 72h. The scratch repair ability of IL-1β-stimulated mesenchymal stem cells has been enhanced(Fig.1A).

To further determine the ability of IL-1β to promote mesenchymal stem cell migration, transwell chamber was performed. As can be seen from the figure, IL-1βcan significantly increase the number of migrated mesenchymal stem cells and migration index, indicating that IL-1βindeed induces migration of mesenchymal stem cells (Fig.1B and C).

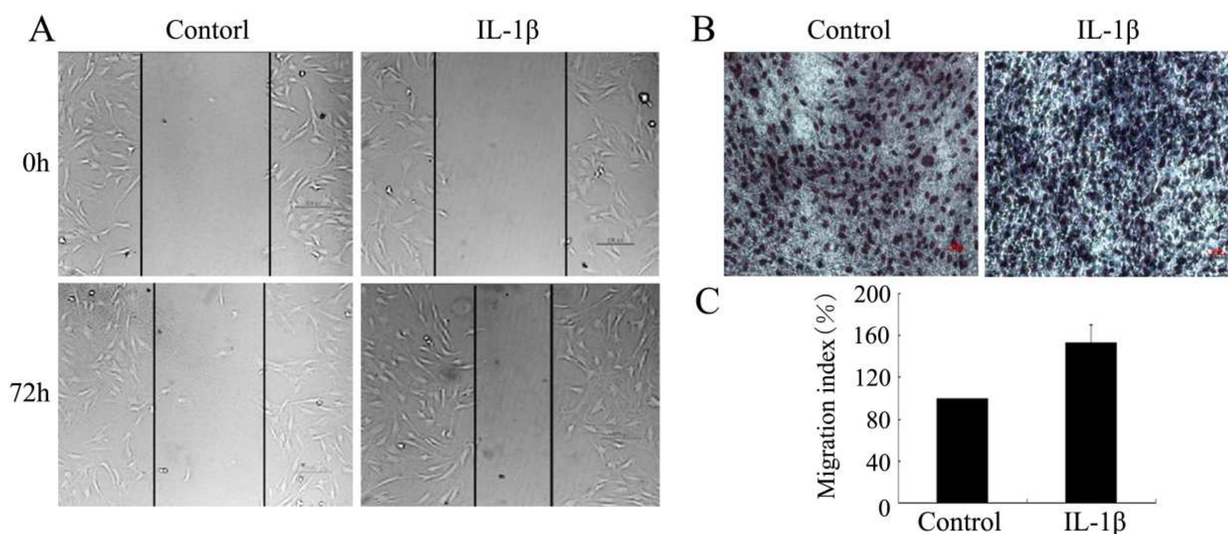

Figure.1IL-1β-induced migration of mesenchymal stem cell. A.IL-1β-induced cell migration in MSCs in wound heal assay(40×). B. Transwell assay in MSCs treated with IL-1β(200×). C. Migration index in MSCs treated with IL-1β

3.2 IL-1β-induced increased the expression of MYL9 and CYR61. In addition, RT-PCR and Western Blot were performed to detect expression of cell migration marker gene CYR61 and MYL9. As shown in Fig. 2, the mRNA and protein levels of CYR61 and MYL9 were increased in IL-1β -stimulated MSCs at 72h (Fig.2).

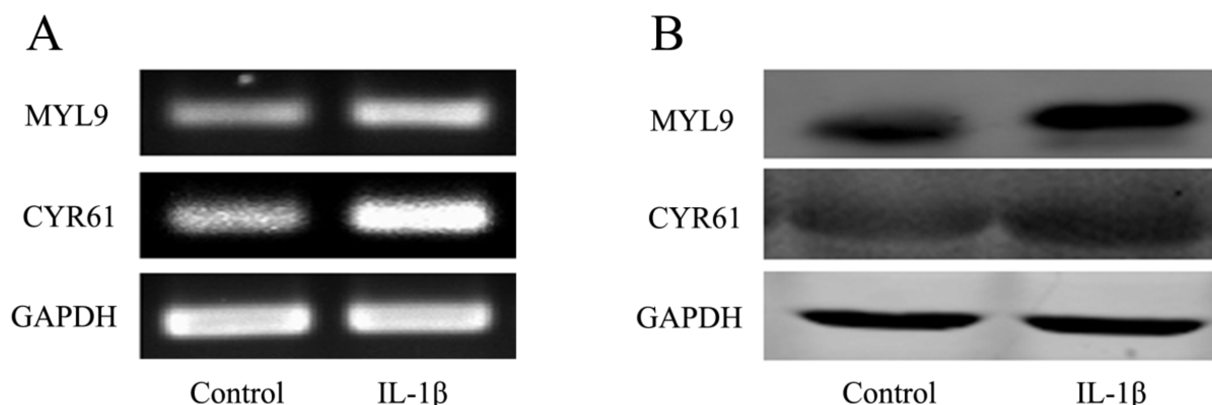

Figure.2 IL-1βincreased the expression of MYL9 and CYR61. A. The mRNA levels of migration markers in MSCs treated with IL-1β. B. The protein levels of migration markers in MSCs treated with IL-1β

3.3 Rho signaling pathway inhibitor suppress IL-1β-induced mesenchymal stem cell migration. Rho signaling pathway has been studied extensively in a variety of cell migration and invasion of tumor cells because of its important role in regulating cytoskeletal protein structure assembly areas. In this study, to detection whether Rho signaling pathway is involved in the process of IL-1β-induced mesenchymal stem cell migration, scratch

wound-healing assay were performed after pretreatment with the inhibitor of Rho/ROCK signaling pathway, Y27632 (10μmol/L) for 0.5 h and then treatment with IL-1β. As shown in Fig. 3A, the scratch repair ability of mesenchymal stem cells pretreated with the inhibitor Y27632 is lower than non-pretreated group, indicating that the migration capacity of mesenchymal stem cells has suppressed by Y27632.

To further determine whether the Rho/ROCK signaling pathway is involved in the migration process of IL-1β-induced mesenchymal stem cells, transwell chamber were performed (Fig.3B). As can be seen from the figure, Rho/ROCK signaling pathway inhibitor Y27632 can significantly decrease the number of migrated mesenchymal stem cells and decrease migration index. Our results show that Rho/ROCK signaling pathway is involved in IL-1β-induced mesenchymal stem cell migration process.

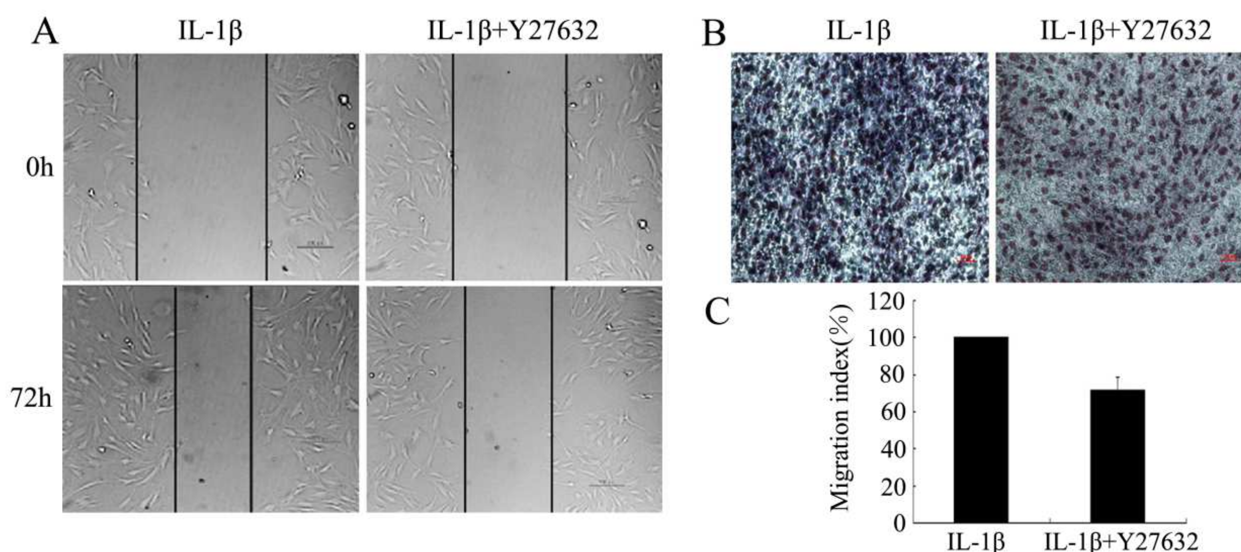

Figure.3 Rho signaling pathway inhibitor suppressed IL-1β-induced mesenchymal stem cell migration. A. Wound heal assay showed that the Rho inhibitor repressed IL-1β-induced cell migration in MSCs (40×). B. Transwell assay showed that the Rho inhibitor repressed IL-1β-induced cell migration in MSCs (200×). C. Migration index in MSCs treated with IL-1β in the presence/absence of Rho/ROCK inhibitor

3.4 Rho signaling pathway inhibitor suppress IL-1β-induced MYL9 and CYR61 expression. Then RT-PCR and Western Blot were performed to detect the expression of cell migration marker gene CYR61 and MYL9 at the mRNA and protein levels. The expression of CYR61 and MYL9 were decreased at the mRNA and protein levels, when cells were pretreated with Rho/ROCK signaling pathway inhibitor Y27632 (Fig.4).

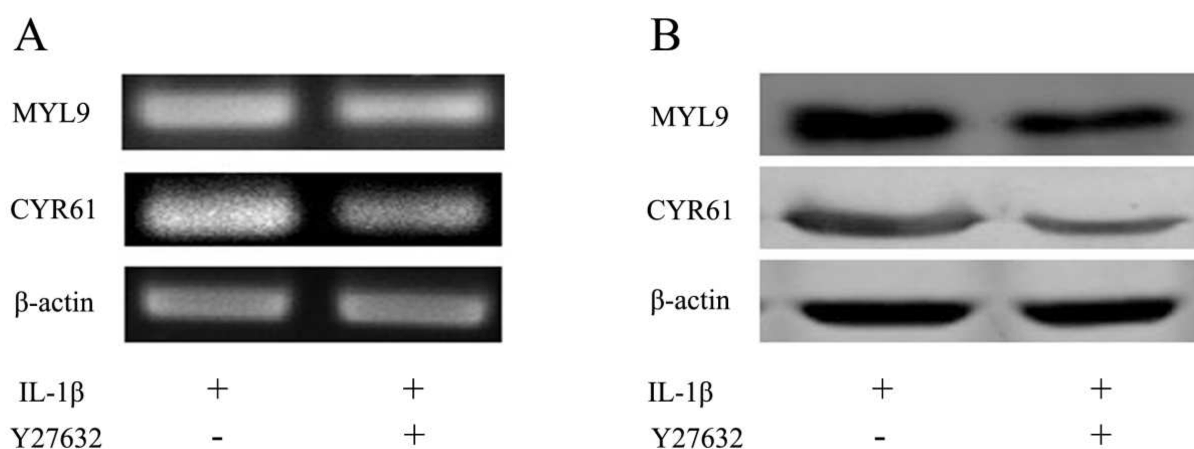

Figure.4 Rho signaling pathway inhibitor suppress IL-1β-induced the expression of MYL9 and CYR61. A. The mRNA levels of migration markers in MSCs treated with IL-1β in the presence/absence of Rho/ROCK inhibitor. B. The protein levels of migration markers in MSCs treated with IL-1β in the presence/absence of Rho/ROCK inhibitor

CONCLUSION

Cell migration is a multifaceted process, involved in the cytoskeleton, matrix environment and signal transduction.

There is a lot of research about role of Rho signaling pathway in mesenchymal stem cell migration, but the results are more controversial. Jaganathan et al. found that physiological activated RhoA can enhance actin stress, inhibit bone marrow mesenchymal stem cell migration, and after suppressed Rho kinase and RhoA activity, increase bone marrow mesenchymal stem cell migration capacity[15]. Chung et al also found that depleted actin can enhance RhoA activity, then suppress bone marrow mesenchymal stem cell migration[16].However, Lee et al showed that Y27632, the inhibitor of Rho/ROCK signaling pathway, can suppress migration of mesenchymal stem cells which is induced by phosphatidic acid LPA[17]. Meriane et al. also showed that inhibition of ROCK activity by Y27632 will completely eliminate the sphingosine phosphate S1P-induced migration of mesenchymal stem cells[18].The Rho signaling pathway in mesenchymal stem cell migration process demonstrated the opposite effect, possibly because mesenchymal stem cell migration involved in different inducing factors, and different inducing factors may not only through the Rho signaling pathway to regulate of mesenchymal stem cells migration. In this paper, we determined that Rho signaling pathway is involved in IL-1β-induced mesenchymal stem cell migration. Of course, further studies about comprehensive and profound dialysis molecular mechanisms about mesenchymal stem cell migration need to be confirmed. These studies will provide a theoretical basis for autologous stem cell repair and cell therapy.

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