CCR2 Contributes to the Homing of MSCs and Liver Regeneration

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Abstract

Objective: Mesenchymal stem cells (MSCs) are emerging as a new therapeutic strategy for liver diseases. However, the inability of stem cells to efficiently return to the target tissue is a major obstacle to repair. The chemokine family and its receptors play an important role in the homing of mesenchymal stem cells. This study was conducted to verify that the overexpression of chemokine receptor CCR2 can contribute to the homing of MSCs, improve the therapeutic effect, and clarify the mechanism of action.

Methods: A mouse model of partial hepatectomy was established. Transcriptome sequencing was used to detect the expression of CCL2 in liver tissue. CCR2 was transfected with lentivirus into BMSCs, and the expression of CCR2 in BMSCs was detected by qRT-PCR. The effect of BMSCs overexpressing CCR2 on liver regeneration in mice with partial hepatectomy was detected by liver index, HE staining and serum ALT and AST content. The migration ability of BMSCs modified by CCR2 was observed by Transwell test and PKH26 staining. The influence of CCL2-CCR2 on the homing signaling pathway of BMSCs was detected by Western blot and scratch experiments.

Results: The expression of CCL2 was up-regulated during the whole process of liver regeneration. BMSCs can express CCR2 receptor, but lentivirus transfected BMSCs can express more CCR2. The BMSCs modified by CCR2 showed stronger migration ability. At 72 hours after partial hepatectomy, the liver index of BMSCS-CCR2 group was higher than that of BMSCs group, the liver tissue structure tended to be normal, and ALT and AST content decreased significantly. Expression of Rho/ROCK signaling pathway protein was increased in the liver of BMSCS-CCR2 group, while cell migration was decreased after treatment with the ROCK inhibitor Y-27632.

Conclusion: In summary, this study suggests that CCR2 expression on BMSCs enhances their targeted migration to the liver of partial hepatectomy mice and promotes liver regeneration. The Rho/ROCK signaling pathway plays an important role in the homing of BMSCs.

Background

The liver is an important organ for metabolism and detoxification. It has a unique dual blood supply, 20 % of the blood comes from the hepatic artery and 80 % from the portal vein. It is rich in food antigens, bacterial products and environmental toxins. Liver is one of the most vulnerable organs in the body due to its anatomical site and functional characteristics. A variety of chemical, physical and biological factors can cause liver damage. Although the liver has a strong regenerative ability, compared with various liver diseases, the self-repair ability of liver cells is far from enough. Therefore, improving the efficiency of liver regeneration is a key step in the treatment of liver diseases.

Mesenchymal stem cells (MSCs), an important member of the stem cell family, originate from the mesoderm and ectoderm. Because of its easy separation, chemotaxis, multidirectional differentiation potential, immunomodulation and other biological characteristics, it has attracted people's attention, and become a popular seed cell in the treatment of various refractory diseases in cell therapy. Homing is a key

step for stem cells to play a role in tissue repair. The inability of stem cells to efficiently homing to the target tissue is a major obstacle affecting the repair effect^[1]. Therefore, improving the homing rate of stem cells is the key to the application of stem cells in disease treatment.

Chemokine families and their receptors not only mediate the migration of white blood cells, but also play an important role in the homing of mesenchymal stem cells^[2]. C-C chemokine ligand 2 (CCL2) is a member of chemokine C-C subfamily. CCL2 is secreted by liver parenchyma cells, Kupffer cells and sinus endothelial cells. Wang et al.^[3] found that 44.6% of MSCs expressed CCL2 receptor CCR2, and the combination of CCL2 and CCR2 promoted the homing of MSCs to ischemic brain tissue. Belema-bedada F et al.^[4] also confirmed that CCR2 can promote the homing of MSCs to the injured heart caused by ischemia/reperfusion. However, the role of CCR2 in promoting MSCs homing remains unclear. Therefore, the study of the role and possible mechanism of CCL2/CCR2 molecular axis in MSCs homing is of great significance for further understanding the mechanism of MSCs promoting liver injury repair and exploring new therapeutic methods for liver injury.

Methods

Production of partial liver resection model in mice

The mice were anesthetized with 10 % chloral hydrate intraperitoneal injection. After abdominal disinfection, the central vertical incision of the lower abdomen of xiphoid process was taken, with a length of 1.5 cm-3 cm, and the epidermis, muscularis and peritoneum were cut successively to fully expose the liver. The middle lobe of the liver and the left lobe were ligated and removed, and the abdomen was closed with sutures successively. After sutures, the incision was disinfected again.

Isolation and culture of bone marrow mesenchymal stem cells

The femur of the mice was taken, and the muscles and fascia were covered with ice packs in a super clean table. Both ends of the femur were cut off with scissors. A syringe was inserted into the medullary cavity, and the bone marrow was washed out, cut and blown into single-cell suspension. Then, DMEM/F12 culture medium containing FBS was added, and conventional culture was performed in an incubator at 37 °C and 5 % CO₂.

Lentivirus transfection of CCR2

The BMSCs growing at log stage in the third generation were selected. When the cells grew to 80 % fusion degree, the CCR2 overexpressed plasmid lentivirus (PGMLV-PA4-CCR2) was used to infect the BMSCs according to the virus infection ratio of 50:1. After infection for 6-8 h, fresh medium was replaced to continue culture. After 48 h, the stable expression strains were screened with puromycin medium with the final concentration of 0.1 mg/L.

Liver index

BMSCs were transferred into partial hepatectomy mice through caudal veins. The weight of mice and the weight of regenerated liver were measured 72, 120 and 168 h after partial hepatectomy, and the liver index was calculated according to the ratio of regenerated liver weight to body weight.

HE staining

Mouse liver tissue was fixed in 4 % paraformaldehyde, embedded in paraffin, and sectioned to 4 μ m thick. Baked at 65 °C for 4.5 h; The sections were dyed with hematoxylin dyeing solution for 3-5 min, washed with tap water, differentiated with differentiation solution, washed with tap water, returned blue solution returned blue, and washed with running water; Sections were dehydrated in 85 % and 95 % gradient alcohol for 5 min, respectively, and then stained in eosin solution for 5 min. The slices were put into anhydrous ethanol I for 5 min, anhydrous ethanol II for 5 min, anhydrous ethanol III for 5 min, dimethyl I for 5min, and xylene II for 5min in sequence. The slices were transparent and sealed with neutral gum. Observe with a light microscope.

Hepatic function detection

The mice in each group were anesthetized by intraperitoneal injection of pentobarbital sodium at 72 h after partial hepatectomy, the abdominal cavity was fixed in supine position, and 1 mL blood was collected from orbit. The mice were left standing at room temperature for 30 min, centrifuged at 4000 r/min for 5 min, and 300 μ L serum was collected. The contents of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were detected by automatic biochemical instrument.

Migration capability of the MSCs

MSCs was vaccinated in the upper chamber (2.0×10^5 / hole), while the lower chamber was fitted with recombinant 100 ng/ml CCL2 for 4 hours, wiped the MSCs, remaining on the upper surface and stained with 0.1 % crystal purple. MSCs migrated to the lower surface were counted microscopically.

Real-time PCR.

Extract 1 μ g RNA from tissue , in the extraction tissue was prepared as described in the Invitrogen reverse recording kit Then the target gene was amplified and PCR tested on PRISM 7900 Sequence Detector by the operating guidelines of QuantiTect SYBR Green RT-PCR Kit (Qiagen), and the copy number of the target gene in the sample calculated the relative content of the target gene from the copy number of β -actin.

Western blot

The liver tissue was cut into pieces, lysate was added into ice to homogenate, supernatant was collected by centrifugation at 4 °C, and protein concentration was measured by BCA method. SDS-PAGE electrophoresis was performed on appropriate protein samples at 80 V for 2 min and then at 120 V for 2 h. 300 mA for 2 h. The PVDF membrane was sealed in 5 % skimmed milk powder for 2 h and

washed with TBST for 3 times, 10 min each time. Primary antibody (1:500 dilution) was incubated at 4 °C overnight and washed with TBST for 3 times, 10 min each time. The secondary antibody (1:2000 dilution) was incubated at room temperature for 1 h, washed 3 times with TBST, 10 min each time, and developed with ECL.

Statistical analysis

SPSS 25.0 statistical software was used to conduct t test on the data of the experimental group and the control group, and $P < 0.05$ was considered statistically significant.

Results

Changes in CCL2 expression after partial liver resection in mice

Mouse regenerated liver tissue 24, 48, 72, 96, 120, 144, 168 h for transcriptome sequencing showed CCL2 expression throughout the liver regeneration (Fig. 1).

Expression of CCR2 in BMSCs

qRT-PCR results showed that BMSCs could express receptor CCR2, the receptor of CCL2, but lentivirus transfected BMSCs could expressed more CCR2(Fig. 2).

Migration ability of the CCR2-modified BMSCs

In vitro Transwell assay showed that MSCs-CCR2 responded to CCL2 stimulation with a higher mobility rate than untransfected MSCs (Fig. 3). 48 h after transfection of BMSCs into CCR2, the cells were stained with PKH26 and transferred into the partially hepatectomy mice through the caudal vein. After 12 h, the right hepatic lobe was removed. Fluorescence microscopy showed that the CCR2-modified BMSCs had a stronger migration ability (Fig. 4).

Effects of overexpressed CCR2 on liver regeneration in partial excision mice

BMSCs overexpressed CCR2 was transferred through the tail vein into part of the hepatectomized mice, At 72 hours after partial hepatectomy, the liver index of BMSCs-CCR2 group was significantly higher than that of the non-transfected BMSCs (Fig. 5). At this time, the hepatic lobule area was larger, the hepatocyte plate was in double rows, hepatocytes were significantly enlarged, and hepatocytes and nuclei were of different sizes. Vacuoles were still visible in the cytoplasm, binuclear hepatocytes and mitosis were visible around the portal area, and stromal cells were active. However, the liver tissue structure of the BMSCs-CCR2 group tended to be normal compared with that of the BMSCs group (Fig. 6), and ALT and AST contents were significantly decreased compared with that of the BMSCs group ($P < 0.05$). (Table 1).

CCL2-CCR2 affects the nesting of BMSCs via the Rho/ROCK signaling pathway

Western blot analysis showed that the expression of Rho/ROCK signaling pathway protein in the liver was enhanced after the CCR2-overexpressed BMSCs were transferred into the partially hepatectomized mice (Fig. 7), while the migration ability of BMSCs treated with ROCK inhibitor Y-27632 was weaker than that of the non-inhibitor treated group (Fig.8).

Discussion

The mouse model of hepatectomy is the best model to study liver regeneration, and can be used to simulate the initiation, proliferation and termination stages of liver regeneration. In 1931, Higgins and Anderson first created the 70 % partial liver resection model in rats, which has been widely used in basic research on liver regeneration, liver tumor, liver failure, liver transplantation and other related researches.

Chemokine CCL2 was first extracted and purified from human glioma cell lines and bone marrow mononuclear cell lines in 1989 [5]. CCL2 gene is located on chromosome 17Q11.2-Q12 and encodes a precursor protein with 99 amino acids. After post-transcriptional modification, it eventually forms a protein with 76 amino acids [6]. CCL2 is secreted by adipocytes, fibroblasts, endothelial cells, vascular smooth muscle cells and other cells, as well as liver parenchyma cells, Kupffer cells and sinus endothelial cells. CCL2 chemotaxis various inflammatory cells such as neutrophils, monocytes, and lymphocytes to the lesion site. Since its discovery, it has been demonstrated to play an important role in inflammatory response, angiogenesis, injury repair, and tumorigenesis^[7,8]

Although the liver has a strong regenerative ability, compared with various liver diseases, the self-repair ability of liver cells is far from enough. Studies have shown that MSCs transplantation contributes to liver damage repair^[9,10]. MSCs transplantation mainly includes two methods: local transplantation and venous transplantation. Local transplantation is a single or multipoint local injection at the site of injury, which may cause local pathological reactions. An venous graft is an injection of stem cells intravenously to the site of injury. The venous route provides a relatively safe mode, but lacks satisfactory homing efficiency for focal targets in vivo. Intravenous injection of mesenchymal stem cells is usually distributed in lung, spleen, kidney, liver, heart and other organs, affecting the therapeutic effect. Therefore, the strategy of strengthening local recruitment will help to improve the effectiveness of cell therapy.

The homing behavior of stem cells to injured or ischemic tissues and the homing mechanism of a series of molecules involved are very similar to the homing mechanism of white blood cells to inflammatory tissues^[11], including the mobilization and migration of MSCs, endothelial adhesion of MSCs and transendothelial migration of MSCs. The interaction between chemokine ligands secreted by damaged tissues and corresponding chemokine receptors expressed on the surface of MSCs is very important for MSCs mobilization^[12]. Andres et al. also demonstrated that transendothelial migration of neural stem cells injected into mouse blood vessels is dependent on CCL2/CCR2 interactions after cerebral ischemia^[13]. In this study, we found that CCL2 is a chemokine ligand that is highly expressed in almost the entire process of liver regeneration. The overexpression of CCR2 in MSCs enhanced the homing ability of MSCs to damaged liver and improved the repair of liver injury.

Rho/ROCK is a universal signal transduction pathway in all tissues of the body, closely related to a variety of biological behaviors such as cell contraction, adhesion, migration, proliferation and cytoskeleton formation^[14]. The key molecules of Rho/ROCK signaling pathway include Rho GTP enzyme, Rho Associated kinase (ROCK) and their acting substrates. Lee et al.^[15] proved that Lysophosphatidic acid (LPA) induction of MSCs migration required RhoA activation, and pretreatment of cells with ROCK inhibitor Y-27632 could significantly inhibit the migration reaction. The binding of CCL2 to its receptor CCR2 can activate the intracellular Rho GTP enzyme^[15]. In this study, it was found that the expression of Rho/ROCK signaling pathway protein in the liver of BMSCs overexpressing CCR2 was enhanced after they were transferred into partially hepatectomy mice, and the migration ability of BMSCs treated with ROCK inhibitor Y-27632 was weaker than that of the non-inhibitor group.

In conclusion, CCL2 was highly expressed after liver injury, and MSCs with CCL2 chemotactic expression migrated and homed to the site of injury through the Rho/ROCK signaling pathway.

Abbreviations

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMSCs: Bone marrow mesenchymal stemcells; CCL2: Chemokine (C-C motif) ligand 2; CCR2: Chemokine (C-C motif) receptor 2; FBS: fetal bovine serum; LPA: lysophosphatidic acid; MSCs: Mesenchymal stem cells; RhoA: Ras homolog gene family member A; ROCK: Rho kinase.

Declarations

Acknowledgements

Not applicable

Authors' contributions Xuekun Xing was a major contributor in writing the manuscript. Jiayang Zhang proposed the conception and performed the data analyses. Runsheng Liu was responsible for the critical work of the manuscript. Hongnan Li and Na Wang corrected the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials Availability of data and materials can be assessed both in the "Methods" section, the "Results" section, and the "Additional files" section.

Ethics approval and consent to participate This study was approved by the Ethics Committee of Laboratory Animals of Guilin Medical University.

Consent for publication Not applicable

Competing interests The authors declare that they have no competing interests.

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Tables

Table 1. Liver function comparison

group	ALT (U · L ⁻¹)	AST (U · L ⁻¹)
BMSCs	198.79±23.48	153.42±20.15
BMSCs-CCR2	135.85±19.68*	89.64±24.35*

*: P <0.05 (compared to the BMSCs group)

Figures

Figure 1

Changes in the expression of the CCL2 gene after partial liver resection in mice

Figure 2

Expression of the CCR2 gene

Figure 3

In vitro migration of MSCs-CCR2 was detected by Transwell invasion experiments

Figure 4

In vivo migration of MSCs-CCR2 was detected by a PHK26 stained fluorescence microscope

Figure 5

Effects of BMSCsCCR2 on the mouse liver index

Figure 6

HE staining shows the effect of BMSCs-CCR2 on the liver structure in mice

Figure 7

Western blot detects the effect of BMSCs-CCR2 on the Rho/ROCK signaling pathway

BMSCs-CCR2+Y-27632

BMSCs-CCR2

Figure 8

The effect of Y-27632 on BMSCs-CCR2 migration ability is observed