**Human umbilical cord mesenchymal stem cells derived Exosomes** **attenuate injury of myocardial infarction by miR-24-3p-promoted M2 macrophage polarization**

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**Supplementary materials**

**Supplementary Methods**

**Peritoneal macrophages isolation**

Peritoneal macrophages were isolated from 8‐week‐old (about weighing 20 g), male C57BL/6 mice by peritoneal lavage that had intraperitoneal injection of 3% thioglycollate 4 days prior, according to a method previously described.14 Briefly, RPMI 1640 medium (Gibco) containing 10% FBS and 1% P/S, was injected into the mouse peritoneal cavity, and peritoneal fluid was collected by a sterile syringe. The collected fluid was filtered through a 70 μm mesh and centrifuged at 250 g for 5 minutes at room temperature. Then cells were re-suspended by medium and cultured for 6 hours, nonadherent cells were discarded and the adherent cells were peritoneal macrophages. The identification of peritoneal macrophages was carried out by flow cytometry analysis with FITC labelled anti-F4/80 body (eBioscience).

**Western blot analysis**

Proteins were isolated from exosomes, cells or tissues using RIPA buﬀer with protease inhibitors (Sigma), and separated by 10% SDS-PAGE (Beyotime) and then transferred to PVDF membranes (Life Technology). After 1 hour of blocking with 5% skim milk at room temperature, the membranes were incubated with primary antibody overnight at 4°C (The dilution ratio of antibodies were listed in Supplemental Table 1) and then incubated with the corresponding secondary antibody for another 1 hour at room temperature. Protein bands were visualized using an ECL chemiluminescence kit (Vazyme) following the manufacturer's instruction.

**Quantitative real‐time PCR (qPCR)**

Total mRNAs were isolated from cells using TRIzol reagent (Invitrogen) and the cDNA was reverse-transcribed using a cDNA Synthesis Kit (Takara). qPCR was performed using the SYBR qPCR Mix Kit (Takara) by ABI Step One-Plus Detection system (Applied Biosystems). The relative expression levels of mRNAs were calculated according to the 2−∆∆Ct method. All primer sequences of mRNAs were listed in Supplemental Table 2.

**Exosomal RNAs or proteins obtain**

To obtain the exosomal RNAs or proteins, the protocol was performed following the method: (1) To get the exosomal proteins by removing RNAs from exosomes, exosome suspension was disposed by five freeze-thaw cycles (-170°C~37°C) and then incubated with 10 μg/mL RNase A (Takara) at 37℃ for 1 hour. Finally, 2000 units/mL RNase A inhibitor (Takara) was added and incubated at 37℃ for an additional hour (the procedure marked in green arrow, Figure 6A). (2) To get the exosomal RNAs by removing proteins from exosomes, exosome suspension was also disposed by five freeze-thaw cycles (-170°C~37°C) and then incubated with 0.5 mg/mL proteinase (Sigma) at 37℃ for 2 hours (the procedure marked in red arrow, Figure 6A).

**RNA deep sequencing analysis**

Total RNA of macrophages was extracted using Trizol Reagent (Invitrogen), 1 μg RNA per sample was used as input material for the RNA sample preparations. First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase, and second strand cDNA synthesis was subsequently performed. PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. At last, PCR products were purified and library quality was assessed on the Agilent Bioanalyzer 2100 system. After cluster generation, the library preparations were performed by BGI Genomics (China). Raw reads of fastq format were firstly processed through in-house perl scripts, and clean reads were obtained by removing reads containing adapter. FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. Differential expression analysis was performed to determine differential expression gene in different groups.

**Supplemental Tables**

**Supplemental Table 1. Primary antibodies used for western blotting**

|  |  |  |  |
| --- | --- | --- | --- |
| Antibody | Company | Host species | Dilution ratio |
| CD63 | Abcam | Mouse | 1:1000 |
| TSG101 | Abcam | Rabbit | 1:1000 |
| CD206 | Santa Cruz | Mouse | 1:1000 |
| Arg1 | Proteintech | Rabbit | 1:1000 |
| iNOS | Abcam | Rabbit | 1:1000 |
| NFκB-P65 | CST | Rabbit | 1:1000 |
| p-P65 | CST | Rabbit | 1:1000 |
| HO-1 | Abcam | Rabbit | 1:1000 |
| VEGFA | Abcam | Mouse | 1:1000 |
| VEGFR2 | Abcam | Rabbit | 1:1000 |
| MMP2 | Abcam | Rabbit | 1:1000 |
| MMP9 | Abcam | Rabbit | 1:1000 |
| Plcb3GAPDH | ProteintechAbcam | MouseMouse | 1:10001:1000 |

**Supplemental Table 2. Primers for qPCR**

|  |  |  |
| --- | --- | --- |
| Gene name | Forward Primer | Reverse Primer |
| Mouse CD206 | TTCAGCTATTGGACGCGAGG | GAATCTGACACCCAGCGGAA |
| Mouse Arg1 | TTGGCTTGCGAGACGTAGAC | GGCCTTTTCTTCCTTCCCAG |
| Mouse iNOS | GGTGAAGGGACTGAGCTGTT | ACGTTCTCCGTTCTCTTGCAG |
| Mouse TNFαMouse 18S | TAGCCCACGTCGTAGCAAACGTAACCCGTTGAACCCCATT | GCAGCCTTGTCCCTTGAAGACCATCCAATCGGTAGTAGCG |

**Supplemental Figure legend**

Supplement Figure 1. UMSCs-Exo promoted angiogenesis and alleviated fibrosis after MI. (A) The interstitial fibrosis of the border area was showed by Sirius red staining at 28 days after MI (Scale bar: 100 μm) and relative quantification of the interstitial fibrosis statistics (n = 4). (B) Neovascularization in the border area was showed by CD31 immunofluorescence staining at 28 days after MI (Scale bar: 200 μm) and relative quantification of the numbers of tubular vessels (n = 4). \**p* < 0.05; \*\**p* < 0.01.

Supplement Figure 2. DiI (red)-labelled UMSCs-Exo localized in the myocardium at 3 days after MI. Scale bar: 200 μm.

Supplement Figure 3. UMSCs-Exo Promoted the polarization of peritoneal macrophages to M2 phenotype in vitro. (A) The morphology of mouse peritoneal macrophages. Scale bar: 200 μm. (B) The identification of peritoneal macrophages was carried out by flow cytometry analysis with FITC labelled anti-F4/80 body. (C) Flow cytometric analysis showed M2 (F4/80+CD206+) and M1 (F4/80+iNOS+) peritoneal macrophages. (D) The percentages of M2 (F4/80+CD206+) phenotype in the peritoneal macrophages (F4/80+). (E) The percentages of M1 (F4/80+iNOS+) phenotype in the peritoneal macrophages (F4/80+). (F) Gene expression of M2 markers (CD206 and Arg1) of peritoneal macrophages. (G) Gene expression of M1 markers (iNOS and TNFα) of peritoneal macrophages. (H) Western blot assay for CD206, Arg1 and iNOS expression of peritoneal macrophages. \**p* < 0.05. \*\**p* < 0.01. \*\*\**p* < 0.001. MΦ: macrophages.