Role of IL-33 in immunoregulation of mesenchymal stem cells

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Abstract: Objective: To isolate and culture mesenchymal stem cells (MSCs) from healthy placenta and detect the biological characteristics of MSCs. To study the immunoregulation function of MSCs and explore the role of IL-33 in the immunoregulation of MSCs. Methods: MSCs were isolated from placenta by enzyme digestion method and their surface antigens were detected by flow cytometry. The IL-33 gene fragment was amplified by RT-PCR and linked to pCDH plasmid. MSCs were infected by lentivirus embedded with the enzyme products detected as positive. T cells and B cells were isolated from peripheral blood by Ficoll density gradient centrifugation. MSCs overexpressed by MSCs and IL-33 were co-cultured with T cells and B cells, respectively. According to Transwell's results, the immune regulation function of MSCs and the role of IL-33 in the immune regulation of MSCs were detected. CONCLUSION: MSCs surface antigens CD105, CD73 and CD90 are positive, CD34, CD45, CD14, CD19 and HLA-DR are negative. MSCs have immunosuppressive function and can inhibit the proliferation of T and B cells. IL-33 has a negative regulatory effect on their immune function.

1. Introduction

Mesenchymal stem cell(MSCs) is a kind of pluripotent progenitor cell, which can differentiate into cells of various tissue lineages such as adipocytes, chondrocytes, osteoblasts, muscle cells, liver cells, etc. In 1970, AJ.Friedenstein first isolated MSCs from bone marrow. After that, MSCs have been confirmed to exist in almost all postpartum organs and tissues [1,2]. MSCs has immunomodulatory function, can escape immune recognition and inhibit immune response, but its molecular mechanism is still being explored. Transforming growth factor (TGF-β1), hepatocyte growth factor (HGF), interferon (IFN-γ), indoleamine 2,3- dioxygenase (IDO) and prostaglandin E2(PGE-2) are considered as cytokines that exert immunomodulatory activity [3]. Interleukin-33 (IL-33), also known as IL-1F11, belongs to the interleukin-1 family (IL-1F) according to the structural homology of cytokines. The gene is located on the short arm of chromosome 9, 9p24.1. It contains eight exons and is more than 42 kb in length. It is usually released by damaged or necrotic barrier cells (endothelial cells and epithelial cells) and serves as a warning signal. It plays an important role in congenital and adaptive immunity and helps tissue homeostasis and response to environmental stress [4, 5]. The study found that MSCs expressed higher level of IL-33. This study mainly explored the immune regulation function of MSCs and the role of IL-33 in the immune regulation of MSCs, providing theoretical basis and reference for the treatment of immune system diseases by MSCs.

2. Materials and Methods

2.1 Material

The samples were collected from the Affiliated Hospital of our college. The pregnant age of GA was 38±2 weeks. After approval by the hospital ethics committee and informed consent of the pregnant women, healthy placental tissue was harvested from the delivery. The reagents and instruments used are: fetal bovine serum (Gibco 16000-044), DMEM (Thermo Fisher High Glucose), Gibco Collagenase (type II), cell filter (Jingan biological hand-held J0070), CO_2 thermostatic incubator (SANYO MCO-5AC), trypsin, fluorescent labeled monoclonal antibody Invitrogen Monoclonal Antibody, pCDH plasmid (Wuhan Miaoling Biology),restriction

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endonuclease XbaI and BamHI-HF (NEB), T4 ligase (NEB), stbl3 receptive cell (Shanghai Weidi Biology), lentivirus (Hanheng Biology), plasmid small extraction kit (QIAGEN), gel Recovery Kit (TIANGEN), PCR instrument (BIORAD), incubator (COYOTE) Electrothermal thermostatic flume (Shanghai Yi Heng Technology), electrophoresis apparatus (BIORAD), high speed refrigerated centrifuge (Thermo Fisher), inverted microscope (Olympus), flow cytometry (BD), gel imaging system (Beijing 61).

2.2 Method

2.2.1 Separation of MSCs

Take about 1cm^3 amniotic membrane tissue specimens, clean them with sterile PBS, cut the tissues and put them into centrifuge tubes, add 5 mL of 0.1% collagen enzyme II (type II), incubate at 37℃ for 30min, 4℃, 1000 rpm/min, centrifuge for 5min, discard the supernatant, and resuspend the cells in 30ml of DMEM(Thermo Fisher High Glucose) medium containing 10% fetal bovine serum. Then the single cell suspension was made by 70 micron disposable aseptic cell filter (Jingan biological handheld J0070). The viable cells were counted by trypan blue staining.

2.2.2 MSCs Culture

The isolated cell suspension was inoculated into a six-well plate at a concentration of 5-10× 10^5/ml.The medium was DMEM (Thermo Fisher High Glucose), and fetal bovine serum (Gibco 16000-044) and double antibody (penicillin) were added. And streptomycin, 100 units/ml),pH =7.3. Under aseptic conditions, the cells were placed in a 5% CO_2 incubator (SANYO MCO-5AC) for primary culture at 37℃, and the cells were observed at regular intervals for good growth, normal morphology and no pollution. Unadhesive cells were removed and the culture medium was updated until the cells grew to confluence. Adherent cells were separated with trypsin (37℃,5 minutes)and subcultured at a ratio of 1: 5.

2.2.3 Detection of Biological Characteristics of MSCs

In 2005, International Society for Cellular Therapy (ISCT) formulated three minimum standards for human MSCs: having the characteristics of plastic adherence in vitro; Has the ability to differentiate into osteoblasts, adipocytes and chondrocytes; Surface antigens CD73, CD90 and CD105 are positive and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules [6, 7]. The surface antigenic properties of MSCs were detected by flow cytometry (BD). Add 20 μl of FITC/PE-labeled monoclonal antibody to 100 μl of cell suspension (cell concentration of approximately 1×10^6 /ml), incubate for 30 minutes in the dark, and then incubate. Add 500 μl PBS and resuspend the machine for analysis.

2.2.4 Cloning and Expression of IL-33 Gene Fragment

IL-33 gene fragment is amplified by RT-PCR, XbaI and BamHI are used as cleavage sites to connect with plasmid pCDH, and the connection product is co-cultured with stbl3 competent cells. A number of monoclonal colonies were selected on the culture plate for PCR, positive results were obtained by gel electrophoresis and sequencing, and then seed culture was carried out. PCDH plasmid containing IL-33 gene fragment was extracted from the culture, and infected MSCs were embedded with lentivirus (pHBAd-MCMV-RFP), and the expression of IL-33 in MSCs was detected by WB.

2.2.5 Detection of MSCs Immune Function

Aseptically, 2ml of peripheral blood was taken and added to RPMI-1640 medium containing PHA, and cultured at 37℃ for 48h. Single nuclear cells were separated by density gradient centrifugation of sucrose-meglumine diatrizoate (Ficoll), and T and B cells were separated by nylon wool separation. Polyamide fibers were evenly filled in polyethylene plastic tubes, soaked in Hanks solution and kept warm. the mononuclear cell suspension was added to the column and placed in a 37℃ incubator for 2h. Pre-heated culture medium containing 10% calf serum was used to wash, and the eluent contained non-adherent T cells. The remaining T cells in the tube were removed by

repeated irrigation several times, and then the plastic tube was extruded while washing with the culture medium. At this time, the eluent contained abundant B cells. Separated T cells and B cells were mixed with MSCs overexpressed by MSCs and IL-33 respectively. T cells and B cells were cultured separately as reference. The immunoregulatory function of MSCs and the role of IL-33 in the immunoregulation of MSCs were detected by Transwell experiment.

3. Result

3.1 Detection of MSCs Surface Antigen

PBS buffer was used instead of antibody to set up blank control, and FITC labeled unrelated antibody was added to the other part as negative control. The selected antibody is Invitrogen Monoclonal Antibody, model number is CD105(SN6),PE, CD73(AD2, PE), CD90(5E10, FITC), CD34 (4H11, FITC), CD45 (RA3-6B2, PE), CD11b (61D3, FITC), CD19 (HIB 19, FITC), HLA-DR (LN3, FITC). After detection, the cell sample showed positive binding to CD105, CD73 and CD90 antibodies and negative binding to CD34, CD45, CD14, CD19 and HLA-DR antibodies (as shown in Fig. 1).

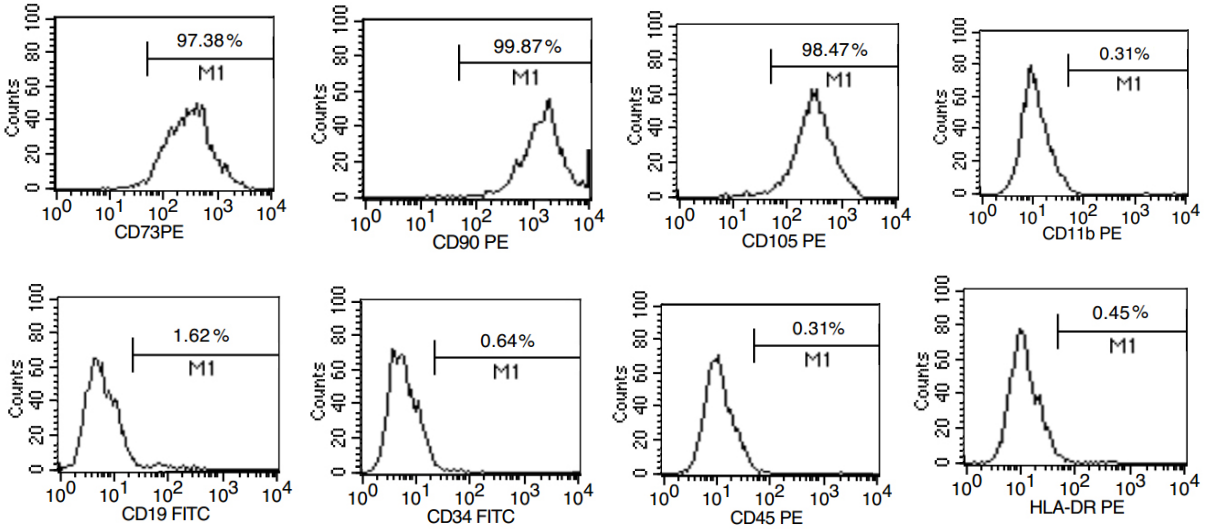


Fig.1. MSCS surface antigen detection results

3.2 Amplification of IL-33 Gene Fragment

Through consulting NCBI and using IL-33 cDNA as template to design primers, the IL-33 gene is amplified. The primer sequence is as follows:

IL-33 F: GCTCTAGAACTGAAAAATGAAGCCTAAAATG;

IL-33 R: CGGGATCCCTAAGTTTCAGAGAGCTTAAACAAG. After 30 cycles of amplification, the target fragment was obtained by electrophoresis (Fig.2)

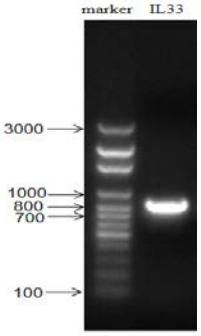


Fig.2. PCR amplification product of il-33

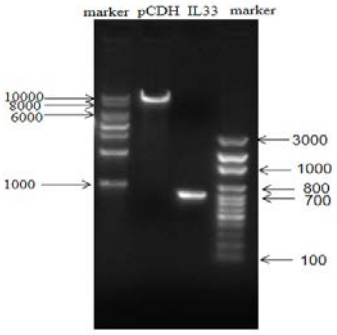


Fig.3.PCDH plasmid and IL-33 enzyme digestion product)

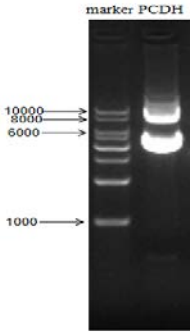


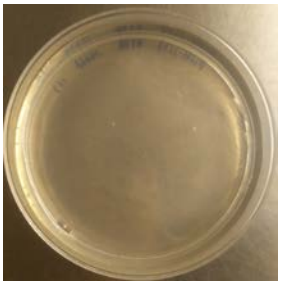
Fig. 4: Plasmid extraction from pCDH

3.3 Enzymatic Ligation of IL-33 to pCDH Plasmid

Interleukin-33 polymerase chain reaction products were recovered by Tiangen Gum Recovery Kit. The pCDH plasmid and IL-33 gum recovery products were digested by enzyme. XbaI and BamHI were selected as restriction sites. The plasmid and target fragments were linked overnight at 1:10 molar ratio with T4 ligase at 16℃, while the products without IL-33 enzyme were used as control group. The concentration of recovered products after enzyme digestion was determined and verified by agarose gel electrophoresis (Fig.3). the concentrations of recovered pCDH and IL33 enzyme digestion product gels were 61ng/μl and 69ng/μl respectively.

3.4 Transfection of IL-33 with pCDH Plasmid Enzymatic Products

10μL of ligation product was added into 100μL of stbl3 competent cells, placed on ice for 30min, heat stimulated at 42℃ for 90s, immediately placed on ice for 2min, added with 900μl LB medium (Amp-), incubated at 37℃ for 1h, coated with Amp+ plate, and incubated overnight at 37℃.After overnight culture, the control group had no single colony, indicating that the plasmid was digested completely without self-connection. The number of colonies in the connecting group was significantly higher than that in the control group, indicating that the recombinant plasmid was successfully transferred into the competent cells (Fig.5). The pCDH plasmid was also extracted from stbl3 using QIAGEN plasmid small extraction kit (Fig.4).



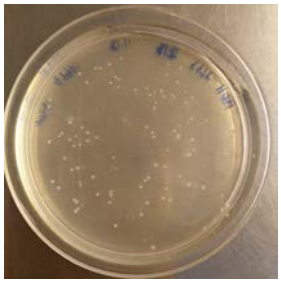


Fig.5. Stbl3 competent cell culture

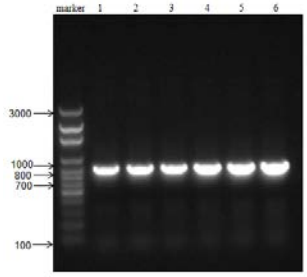


Fig.6. PCR of IL-33 monoclonal colony

3.5 Monoclonal PCR Identification

Six monoclonal antibodies were selected from the plate for culturing stbl3 competent cells and inoculated into 1ml LB medium (Amp+) and cultured at 37℃ until the bacterial solution was turbid. 1μl of bacterial solution was taken for PCR identification of bacterial solution. The results showed that all 6 single colonies were positive clones (Fig.6). the colonies identified as positive by PCR were sent to Jin weizhi for sequencing, with a size of 821bp, which was consistent with the expected size.

3.6 Interleukin-33 Lentivirus Embedded Transfection into MSCs

Adherent MSCs were spread in a 6-well plate at a rate of 1×10^5/ well for 24 hours. The original medium was replaced by 2ml of fresh medium containing 6 μ g/ml of Polybrene. A suiTable amount of lentivirus (Hanheng Biology, pHBAd-MCMV-RFP) suspension was added and cultured at 37℃ for 48 hours. IL-33 was successfully expressed in MSCs by WB detection.

3.7 Immune Function of MSCs and Effect of IL-33

Through Transwell experiment, MSCs can inhibit the proliferation of T cells in mixed lymphocyte culture (MLC). The inhibition of MSCs on T cells may be influenced by soluble factors secreted by MSCs rather than by inducing apoptosis, which is also illustrated by the fact that the inhibited T cells do not undergo apoptosis and proliferate effectively when stimulated again [8,9].MSCs can also inhibit the proliferation and differentiation of B lymphocyte, and the secretion of IgM, IgG and IgA is also impaired. Its molecular mechanism is mainly due to the physical contact between MSCs and B cells and the soluble factors released by MSCs in culture medium, which results in the blockade of B cells in the phase of G_0/G_1 cell cycle [10], but MSCs overexpressed by IL-33 can promote it. In addition, the secretion of IgM, IgG and IgA increased

with the proliferation of B cells (Fig.7), indicating that IL-33 played a negative role in the immune regulation of MSCs.

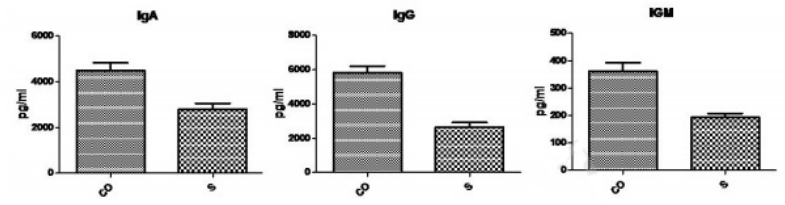


Fig.7. co is the co-culture group of IL-33 overexpressed MSCs and b cells, and s is the separate culture group of b cells

4. Discussion

MSCs is an easy-to-culture pluripotent cell with immunomodulatory properties and has a wide range of potential clinical applications, including tissue repair, delivery of drugs or genes to diseased tissues or organs, improvement of BM implantation and inhibition of GVHD. However, there are also safety concerns in its application, including tumorigenicity, ectopic tissue formation and differential expression in vitro and in vivo cultures. In vitro cell culture characteristics cannot fully represent the in vivo characteristics of cells and may be misleading [11]. The immunological results produced by MSCs in vivo are not completely predicTable and even show conflicting results. It is now generally accepted that the immunomodulatory properties of MSCs are not constitutive but are induced by various media present in the inflammatory microenvironment. .

IL-33 acts as an “alarm” signal in innate immunity, inflammation and allergic reactions, and initiates myeloid differentiation factor 88 (MyD88) dependence by binding to the ST2 / IL-1RAcP receptor complex expressed in target cells. The signaling pathway activates lymphocytes to participate in immune responses [12]. Studies have shown that IL-33 overexpressing MSCs can promote the proliferation of B cells, and the secretion of IgA, IgG and IgM also increases, indicating that IL-33 may have a negative regulatory effect on the immunosuppressive function of MSCs.However, it is not easy to understand the immunoregulation mechanism of MSCs, because the molecular process driving MSCs to react is very complex, and the cell dose also has an important influence on the function of MSCs. The difference in immunoregulation of MSCs is not only affected by the interaction between cells, but also related to the microenvironment in which they are located [12]. Therefore, the immunoregulatory function of MSCs and the role of IL-33 in regulation need further study. The plasticity of MSCs immunoregulatory properties may have important guiding significance in clinical application.

Acknowledgements

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