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Hypoxic conditioned medium of placenta-derived mesenchymal stem cells protects against scar formation

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ABSTRACT

Aims: Scar formation after wound repair affects people's daily life. Mesenchymal stem cells were reported to have a beneficial role in attenuating the scar formation. In the present study, placenta-derived mesenchymal stem cells (PMSCs) were isolated and the effects of hypoxic conditioned medium of PMSCs on scar formation were explored.

Main methods: To evaluate the effect of hypoxia on PMSCs, proliferation of PMSCs was detected by trypan blue staining and the HIF-1α level was detected by western blot. Then in vivo scar formation assay was performed and the histopathologic changes were evaluated by HE staining and levels of TGF-β1 and collagen I were detected by quantitative real-time PCR. The IL-10 level was detected by ELISA and then migration of HFF-1 cells was detected by wound healing assay after treatment with IL-10 or IL-10 antibody.

Key findings: Our study showed that hypoxic conditioned medium of PMSCs reduced scar formation in vivo and inhibited the proliferation and migration of skin fibroblasts in vitro. Further mechanism study showed that, the level of IL-10 was affected by hypoxia, treatment with IL-10 mimicked the function of hypoxic conditioned medium of PMSCs and inhibition of IL-10 reversed the protective role of hypoxic conditioned medium of PMSCs. Thus, hypoxic conditioned medium of PMSCs may perform the protective role against scar formation through IL-10.

Significance: Our study reveals a possible mechanism of the protective effect of PMSCs against scar formation and provides evidence for the hypothesis that PMSCs may be a promising therapy for the treatment of wounds.

1. Introduction

Wound healing is a complicated process that involves inflammation, cell proliferation, differentiation, migration, angiogenesis and remodeling of extracellular matrix (ECM) [1]. Wound healing requires the cooperation of multiple cells, cytokines and extracellular matrix proteins [1]. However, excessive wound repair results in hypertrophic scar or keloid scar [2,3]. In the process of scar formation, skin fibroblast plays important roles. Fibroblast can migrate to the damaged areas and produce matrix to restore the structure of the damaged areas [1].

Mesenchymal stem cells (MSCs) have the properties of stem cells. MSCs are reported to have beneficial effects on the treatment of wounds [4–6]. MSCs can migrate to the injured areas in response to the chemotactic signals, differentiate into skin cells, regulate the inflammatory response, repair the damaged tissues, and facilitate tissue regeneration through paracrine signaling [1]. Bone marrow-derived mesenchymal stem cells (BMSCs) are well-known MSCs. Although the outcomes of BMSCs on wound repair are wonderful, BMSCs accounts for 0.001%–0.01% of the nucleated cell population in the bone marrow of adults [7], it's hard to harvest and with invasive procedures.

Placenta-derived mesenchymal stem cells (PMSCs) are a kind of MSCs harvested from placentas. PSMCs show characteristics similar to BMSCs [8]. A recent report reveals that PMSCs also show excellent effects on wound healing [7]. Placentas are discarded generally after birth as medical wastes. In placentas, the content of PMSCs is abundant, and PMSCs are easy to harvest without invasive procedures. The use of PMSCs as therapy does not cause ethical issues [9]. PMSCs showed protective effects on wound repair [7], however, the effects of PMSCs on scar formation are still not clear.

In the present study, the effects of PMSCs on scar formation in normoxic condition and hypoxic condition were explored. The effects of normoxic and hypoxic conditioned medium of PMSCs on scar formation were explored in vivo and the influences of normoxic and hypoxic conditioned medium of PMSCs on migration of skin fibroblasts were detected in vitro. Our study revealed that hypoxic conditioned medium of

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PMSCs performed a protective effect against scar formation and PMSCs may become a promising therapy for wounds.

2. Materials and methods

2.1. Isolation of PMSCs

Placental tissues of cesarean full-term healthy newborns were obtained under sterile conditions. PMSCs were isolated as described previously [10]. Briefly, the placental tissues were washed with PBS and the tunica adventitia was removed. After washing with PBS, the placental tissues were cut into small pieces and attached to the bottoms of culture flasks procoated with fetal bovine serum (FBS) (Hyclone, Logan, UT, USA). The flasks were then cultured in a 37 °C incubator with 5% CO_2. 12 h latter, Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Grand Island, NY, USA) with 10% FBS was added into the culture flasks. After the cells moving out of the tissue pieces, PMSCs were harvested and seeded into 6-well plates for subsequent culture. The isolated PMSCs were grown in DMEM with 10% FBS and maintained in a humid atmosphere at 37 °C with 5% CO_2. When the cell confluence reached 80–90%, cells were harvested by digestion with trypsin tyrosine and seeded in new plates. This study was approved by the Ethics Committee of China Medical University.

2.2. Identification of the isolated PMSCs

The isolated PMSCs were identified by flow cytometry with CD34, CD45, CD73, CD90, CD105 and HLA-DR staining. Cells were harvested and resuspended in PBS. Then cells were incubated with fluorescein isothiocyanate-conjugated antibodies against CD34, CD45, CD73, CD90, CD105 and HLA-DR (eBioscience, San Diego, CA, USA) respectively, at room temperature for 30 min in the dark. Then the isolated PMSCs were analyzed with flow cytometry (BD, Franklin Lakes, NJ, USA).

2.3. Cell culture

The isolated PMSCs were seeded in 6-well plates with 1×10^5 cells in each well. After attaching to the well, the cells were cultured in normoxic condition (20% O_2, 5% CO_2) or hypoxic condition (5% or 1% O_2, 5% CO_2) respectively for 72 h. Then cells and the conditioned medium were collected for the subsequent experiments. Human skin fibroblasts HFF-1 were obtained from Type Culture Collection Center of Chinese Academy of Science (Shanghai, China). HFF-1 cells were grown in DMEM medium supplemented with 10% FBS and cultured in an incubator of 37 °C with a humid atmosphere and 5% CO_2.

2.4. Animal experiment protocol

30 C57BL/6 mice (8-week old and weighing 20–25 g) were obtained from Vital River Laboratory Animal Technology Co. Ltd., (Beijing, China). Mice were divided into 3 groups ($n = 10$ for each group) and anesthetized with 10% chloral hydrate (3.5 ml/kg, intraperitoneal injection). The dorsal surfaces of the trunks of mice were shaved and a 2 cm-diameter scald was made on the back of each mouse. Briefly, mice were secured with a 2 cm-diameter plastic container. 2 ml hot water (80 °C) was injected into the plastic container. The dorsal surfaces of mice were contacted with hot water for 10 s. Thereafter, mice were placed in individual cages. After the scald model was established, 100 μl normal medium, normoxic conditioned medium, or hypoxic conditioned medium of PMSCs was injected (subcutaneous injection) into the skin tissues around scald areas. Images of mice were captured on day 8 and day 15. The contracture rate was calculated using the following formula: Contracture rate = wound size on specific day/wound size on initial day × 100%. 15 days later, the mice were sacrificed and the scald tissues were collected for HE staining and quantitative real-time PCR (qRT-PCR). All animal experiments were performed according to Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committees of China Medical University

2.5. Trypan blue staining

PMSCs were cultured in normoxic or hypoxic condition for 72 h. Then trypan blue was added into cells of each group. The number of cells without staining with trypan blue was counted. HFF-1 cells were treated with normal medium, normoxic conditioned medium or

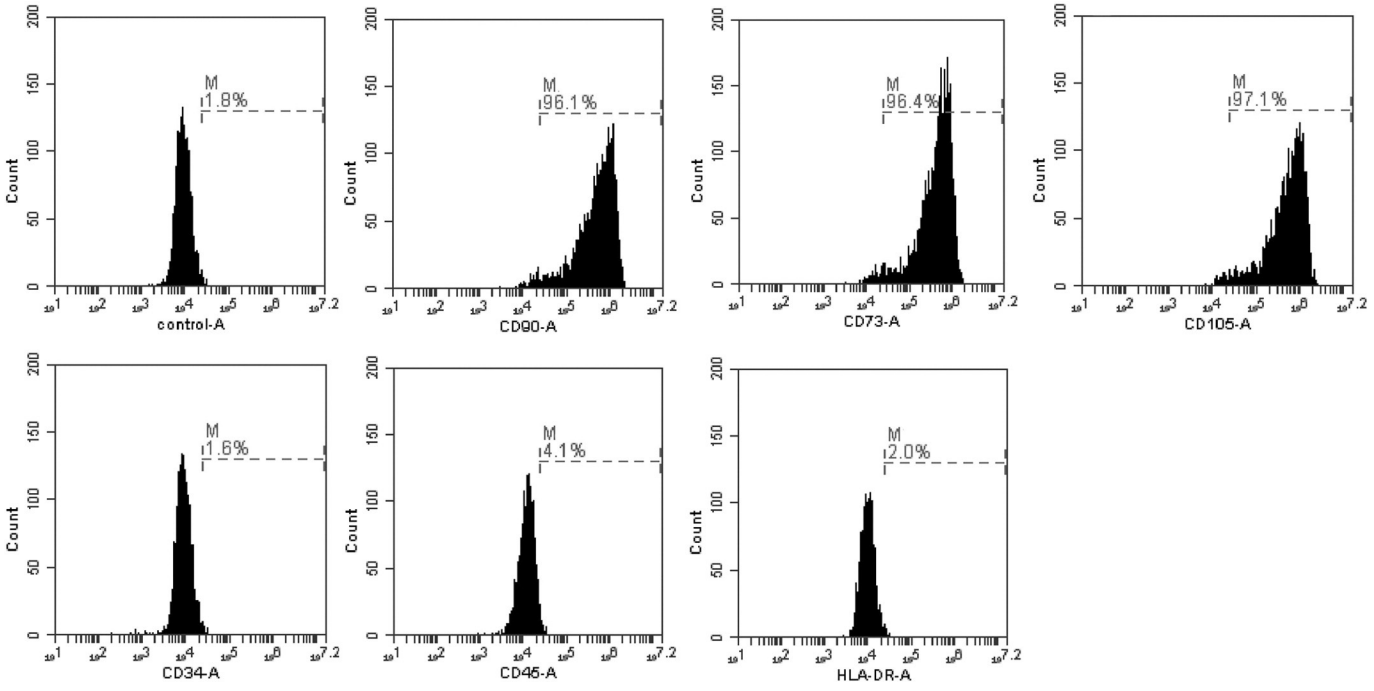

Fig. 1. Identification of the isolated PMSCs. The isolated PMSCs were identified by flow cytometry with CD34, CD45, CD73, CD90, CD105 and HLA-DR.