Hypoxia modulates cell migration and proliferation in placenta-derived mesenchymal stem cells

Li Li, MSc,[a] Prashant Kumar Jaiswal, PhD,[b] Georges Makhoul, MD, MSc,[a] Rishi Jurakhan, MSc,[a] Kaviyanka Selvasandran, MSc,[a] Khalid Ridwan, MD, MSc,[a] and Renzo Cecere, MD[a,b]

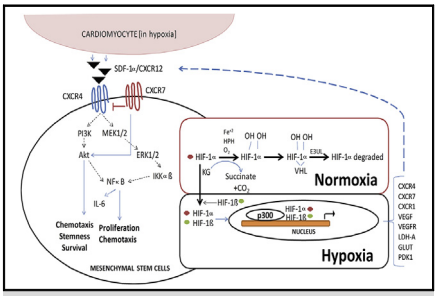
ABSTRACT

Objectives: For more than a decade, stem cells isolated from different tissues have been evaluated in cell therapy. Among them, the human bone marrow–derived mesenchymal stem cells (hBM-MSCs) were investigated extensively in the treatment of myocardial infarction. Recently, the human placenta–derived mesenchymal stem cells (hPD-MSCs), which are readily available from a biological waste, appear to be a viable alternative to hBM-MSCs.

Methods: C-X-C chemokine receptor type 4 (CXCR4) gene expression and localization were detected and validated in hPD-MSCs and hBM-MSCs via polymerase chain reaction and immunofluorescence. Subsequently, cell culture conditions for CXCR4 expression were optimized in stromal-derived factor-1 alpha (SDF1-α), glucose, and cobalt chloride ($CoCl_2$) by the use of cell viability, proliferation, and migration assays. To elucidate the cell signaling pathway, protein expression of CXCR4, hypoxia-inducible factor-1α, interleukin-6, Akt, and extracellular signal-regulated kinase were analyzed by Western blot. CXCR4-positive cells were sorted and analyzed by florescence-activated cell sorting.

Results: CXCR4 was expressed on both hPD-MSCs and hBM-MSCs at the basal level. HPD-MSCs were shown to have a greater sensitivity to SDF-1α–dependent cell migration compared with hBM-MSCs. In addition, CXCR4 expression was significantly greater in both hPD-MSCs and hBM-MSCs with SDF-1α or $CoCl_2$-induced hypoxia treatment. However, $CXCR4^+$ hPD-MSCs population increased by 10-fold in $CoCl_2$-induced hypoxia. In contrast, only a 2-fold increase was observed in the $CXCR4^+$ hBM-MSCs population in similar conditions. After $CoCl_2$-induced hypoxia, the CXCR4/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase signaling pathway was activated prominently in hPD-MSCs, whereas in hBM-MSCs, the CXCR4/phosphatidylinositol 3-kinase/Akt pathway was triggered.

Conclusions: Our current results suggest that hPD-MSCs could represent a viable and effective alternative to hBM-MSCs for translational studies in cardiocellular repair. (J Thorac Cardiovasc Surg 2017;154:543-52)



Human placenta–derived mesenchymal stem cells have a greater sensitivity to SDF-1α–dependent cell migration than human bone marrow–derived mesenchymal stem cells.

Central Message

Greater sensitivity of human placenta–derived mesenchymal stem cells to stromal-derived factor-1 alpha–dependent cell migration compared with human bone marrow–derived mesenchymal stem cells indicates that they could be used as alternative to human bone marrow–derived mesenchymal stem cells for experimental studies.

Perspective

Our data provide new insights into the comparative molecular mechanisms that regulate mesenchymal stem cell (MSC) migration derived from different tissue sources (bone and placenta). Human placenta–derived MSCs were shown to have greater sensitivity to stromal-derived factor-1 alpha–dependent cell migration compared with human bone marrow–derived MSCs. These findings may have experimental implications for the use of human placenta–derived MSCs as an alternative to human bone marrow–derived MSCs.

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From the [a]Division of Experimental Surgery, McGill University; and [b]Division of Cardiac Surgery, McGill University Health Centre, Montreal, Quebec, Canada.

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Address for reprints: Renzo Cecere, MD, Glen Campus - The Royal Victoria Hospital, McGill University Health Center, 1001 Decarie Blvd, Block C, C07.1284, Montreal, Quebec H4A 3J1, Canada (E-mail: renzo.cecere@muhc.mcgill.ca).

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Abbreviations and Acronyms

$CoCl_2$	= cobalt chloride
CXCR4	= C-X-C chemokine receptor type 4
DMEM	= Dulbecco's modified Eagle's medium
ERK	= extracellular signal-regulated kinase
FACS	= fluorescence-activated cell sorting
hBM-MSCs	= human bone marrow–derived mesenchymal stem cells
hPD-MSCs	= human placenta–derived mesenchymal stem cells
IL-6	= interleukin-6
MEK	= mitogen-activated protein kinase kinase
MI	= mycocardial infarction
MSC	= mesenchymal stem cell
NFκB	= nuclear factor $\kappa\beta$
RT-PCR	= reverse transcription polymerase chain reaction
SDF-1α	= stromal-derived factor-1 alpha

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Myocardial infarction (MI) is one of the leading causes of death worldwide.[1] It triggers a series of histologic, cellular, and molecular modifications resulting in a plethora of unfavorable changes to the myocardium.[2] Adding to this is the limited regenerative capacity of the adult cardiac myocytes as the result of their permanent cell-cycle arrest.[3] Altogether, an increasing concern exists regarding the absence of a robust remedy that could reverse the pathophysiologic progression after MI.

Mesenchymal stem cells (MSCs) have the capacity to self-renew and differentiate into various other cell lineages, including cardiomyocyte-like cells, which can integrate into the native tissue.[4-7] MSCs paracrine secretions also have been reported to be beneficial for cardiac repair partly through preserving the extracellular matrix homeostasis.[2,8] Despite such intriguing properties, the use of MSCs in clinical trials for the treatment of MI has had modest success, mostly related to low engraftment rates, short-term homing, and impaired functional activity.

Stem cells isolated from the bone marrow are the most commonly accessed source. These human bone marrow–derived mesenchymal stem cells (hBM-MSCs) have been studied extensively for the treatment of MI.[9] Interestingly, MSCs additionally were reported in other tissues, such as the umbilical cord, the adipose tissue, the liver, and the term placenta. With its easy accessibility, the human placenta generally is discarded after birth, and the stem cells can be readily isolated, avoiding any ethical concerns.[10] Moreover, few studies have demonstrated that the human placenta–derived mesenchymal stem cells (hPD-MSCs) could be a suitable alternative to hBM-MSCs in cardiac repair.[11]

After MI, the stromal-derived factor-1 alpha (SDF-1α) and its chemokine receptor C-X-C chemokine receptor type 4 (CXCR4) is a critical axis in regulating cell migration, homing, and engraftment. It has been shown that SDF-1α is expressed and secreted by cardiomyocytes of the ischemic heart, whereas CXCR4 is expressed on the cell surface of MSCs.[12] The interaction between these 2 components elicits a migratory response of the MSCs up the SDF-1α gradient, and this mechanism assists these stem cells in reaching the site of infarction.

The CXCR4/SDF1-α axis triggers a downstream signaling cascade tightly regulated by various pathways, such as the phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinase pathways.[13-15] In the PI3K/Akt pathway, Akt is responsible for phosphorylating many cytosolic and nuclear molecules involved in cell survival, cell-cycle progression, and cell growth.[16] In the mitogen-activated protein kinase pathway, nuclear factor $\kappa\beta$ (NFκB) and other transcription factors relevant to cardiac repair are activated.[2,17]

Despite their cardiac repairing properties and their paracrine effects, the hPD-MSCs have not been well explored as the hBM-MSCs, in particular at the SDF-1α/CXCR4 axis. Thus, this study aimed to assess the appropriateness of hPD-MSCs as a novel alternative cell source for cardiac repair.

MATERIALS AND METHODS

Cell Culture and Treatment

The hPD-MSCs (kindly donated by Dr Huang Yen laboratory in Taiwan[18]) and hBM-MSCs (Lonza, Basel, Switzerland) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Cells at confluence were harvested and seeded onto 10-cm cell culture dishes (Nest Scientific USA, Rahway, NJ). Cells were treated under different conditions: SDF-1α (PeproTech, Rocky Hill, NJ), glucose (Sigma-Aldrich, St Louis, Mo), and cobalt chloride ($CoCl_2$; Sigma-Aldrich) for 12 hours followed by incubation at 37°C in humidified 5% CO_2 conditions.

Immunofluorescence

1×10^4 of hPD-MSCs and hBM-MSCs were seeded on a 24 × 55-mm microscope cover glass in 6-cm cell-culture dishes. Subsequently, cells were fixed with ice-cold acetone, blocked with 2% bovine serum albumin,