ISSN 1948-0210 (online)

World Journal of *Stem Cells*

World J Stem Cells 2022 September 26; 14(9): 684-743

Published by Baishideng Publishing Group Inc

World Journal of Stem Cells

Contents

Monthly Volume 14 Number 9 September 26, 2022

REVIEW

ORIGINAL ARTICLE

Basic Study

World Journal of Stem Cells

Monthly Volume 14 Number 9 September 26, 2022

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Stem Cells*, Huai-Yong Chen, PhD, Director, Professor, Tianjin Key Laboratory of Lung Regenerative Medicine, Tianjin Institute of Respiratory Diseases, Haihe Hospital, Tianjin University, Tianjin 300350, China

AIMS AND SCOPE

The primary aim of *World Journal of Stem Cells* (*WJSC*, *World J Stem Cells*) is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, *etc*.

INDEXING/ABSTRACTING

The *WJSC* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Journal Citation Reports/Science Edition, PubMed, PubMed Central, Scopus, Biological Abstracts, BIOSIS Previews, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 Edition of Journal Citation Reports cites the 2021 impact factor (IF) for *WJSC* as 5.247; IF without journal self cites: 5.028; 5-year IF: 4.964; Journal Citation Indicator: 0.56; Ranking: 12 among 29 journals in cell and tissue engineering; Quartile category: Q2; Ranking: 86 among 194 journals in cell biology; and Quartile category: Q2. The *WJSC*'s CiteScore for 2021 is 5.1 and Scopus CiteScore rank 2021: Histology is 17/61; Genetics is 145/335; Genetics (clinical) is 42/86; Molecular Biology is 221/386; Cell Biology is 164/274.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Stem Cells

ISSN
ISSN 1948-0210 (online)

LAUNCH DATE
December 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Shengwen Calvin Li, Carlo Ventura

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-0210/editorialboard.htm

PUBLICATION DATE
September 26, 2022

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2022 September 26; 14(9): 684-699

DOI: 10.4252/wjsc.v14.i9.684

ISSN 1948-0210 (online)

REVIEW

Strategies to improve the effect of mesenchymal stem cell therapy on inflammatory bowel disease

Meng-Yue Shi, Lian Liu, Fu-Yuan Yang

Specialty type: Cell and tissue engineering

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B, B
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Amin A, United Arab Emirates; Hassaan NA, Egypt; Kamalabadi-Farahani M, Iran; Prasetyo EP, Indonesia; Song BW, South Korea

Received: May 18, 2022
Peer-review started: May 18, 2022
First decision: June 23, 2022
Revised: July 7, 2022
Accepted: September 8, 2022
Article in press: September 8, 2022
Published online: September 26, 2022

Meng-Yue Shi, School of Medicine, Yangtze University, Jingzhou 434023, Hubei Province, China

Lian Liu, Department of Pharmacology, Medical School of Yangtze University, Yangtze University, Jingzhou 434023, Hubei Province, China

Fu-Yuan Yang, Health Science Center, Yangtze University, Jingzhou 434020, Hubei Province, China

Corresponding author: Fu-Yuan Yang, PhD, Lecturer, Health Science Center, Yangtze University, No. 1 Xueyuan Road, Jingzhou 434020, Hubei Province, China.
ivansblue@sina.com

Abstract

Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis and is an idiopathic, chronic inflammatory disease of the colonic mucosa. The occurrence of IBD, causes irreversible damage to the colon and increases the risk of carcinoma. The routine clinical treatment of IBD includes drug treatment, endoscopic treatment and surgery. The vast majority of patients are treated with drugs and biological agents, but the complete cure of IBD is difficult. Mesenchymal stem cells (MSCs) have become a new type of cell therapy for the treatment of IBD due to their immunomodulatory and nutritional functions, which have been confirmed in many clinical trials. This review discusses some potential mechanisms of MSCs in the treatment of IBD, summarizes the experimental results, and provides new insights to enhance the therapeutic effects of MSCs in future applications.

Key Words: Mesenchymal stem cells; Inflammatory bowel diseases; Inflammation; Pretreatment; Gene editing

Core Tip: Mesenchymal stem cell (MSC) transplantation is a novel treatment method for inflammatory bowel disease (IBD) that has exhibits certain achievements in clinical trials. Here, we reviewed the developed strategies for enhancing the therapeutic effect of MSCs, and among these, pretreatment with MSCs is the most common method. The pretreatments include bioactive substances, hypoxia and modification of culture methods and are able to enhance the migration ability of MSCs to repair the damaged intestinal mucosa or upregulate the expression of cytokines. These methods provide new ideas for the future clinical application of MSCs in the treatment of IBD.

Citation: Shi MY, Liu L, Yang FY. Strategies to improve the effect of mesenchymal stem cell therapy on inflammatory bowel disease. *World J Stem Cells* 2022; 14(9): 684-699
URL: https://www.wjgnet.com/1948-0210/full/v14/i9/684.htm
DOI: https://dx.doi.org/10.4252/wjsc.v14.i9.684

INTRODUCTION

Inflammatory bowel disease (IBD) is divided into three types: Chronic IBD, ulcerative colitis (UC) and Crohn's disease (CD). Genetic susceptibility, environmental factors, the intestinal microbiota and the immune system play important roles in the pathogenesis of IBD[1], and immune factors may be the most direct and important factor[2]. In recent decades, the incidence rate of IBD has increased worldwide. The increase in the incidence of IBD has led to increased social and economic burdens[3]. IBD is traditionally considered common in the Western world. However, data from the last ten years showed that the IBD incidence rates in newly industrialized countries, including China and India, are increasing[4,5]. IBD has developed into a common disease of the digestive system in China. Patients with IBD may exhibit extracolonic manifestations, such as primary sclerosis cholangitis and arthritis, and are also more prone to complications, such as colon cancer, coronary artery disease, osteoporosis and venous thrombosis, than the general population[6,7]. The routine clinical treatment for IBD includes three categories: Traditional therapeutic drugs, biological agents and new small-molecule drugs. The traditional therapeutic drugs include 5-aminosalicylic acid (5-ASA), glucocorticoids such as budesonide and immunosuppressants such as azathioprine (AZA), 6-mercaptopurine (6-MP), ciclosporin and methotrexate. Anti-tumor necrosis factor (TNF)-α drugs, insulin receptor antagonists, and interleukin (IL) inhibitors, are biological agents widely used for the treatment of IBD. The new small-molecule drugs include selective Janus kinase inhibitors, and sphingosine-1-phosphate receptor modulators[8,9]. Although these drugs alleviate IBD, maintaining the effects is difficult, and the expected effect is not ideal. More importantly, these drugs may lead to various adverse reactions[10], such as a risk of increased mortality. The use of corticosteroids has been shown to be associated with skin effects, weight gain, hyperglycaemia, osteoporosis, adrenal insufficiency and cataracts. The use of immunosuppressants also increase the risk of opportunistic infection. Intolerance or the potential occurrence of bone marrow/liver toxicity caused by immunomodulators may result in treatment cessation in one quarter of patients[11]. Ileocolectomy is the most common surgical strategy used to treat patients with CD, but this treatment rarely cures the disease; new lesions usually develop rapidly at the anastomosis, and a risk of urinary incontinence has been documented[12,13]. Therefore, new treatments are needed to improve this condition without a risk of incontinence. The new era of cell-based therapy in stem cell biology has provided promising prospects and aroused great interest from scientists, clinicians and patients[14]. Mesenchymal stem cells (MSCs) are heterogeneous spindle-shaped cells with the ability to differentiate into osteoblasts, chondrocytes and adipocytes *in vitro*. MSCs originate from various tissues, including the bone marrow, umbilical cord, placenta, fat and tooth tissue[15]. Considering their immunoregulatory and nutritional characteristics, MSCs have become promising candidates for the treatment of autoimmune diseases and have promoted the development of regenerative medicine. Inflammatory signals stimulate bone marrow MSCs (BMMSCs) to produce a variety of growth factors, that accelerate tissue repair by promoting angiogenesis, extracellular matrix remodeling and tissue progenitor cell differentiation. BMMSCs effectively regulate immune cells in the inflammatory microenvironment. Interestingly, the immunomodulatory effects of BMMSCs are not inherent but are determined by the type and intensity of the inflammatory reaction[16]. In this review, we discuss the research progress and possible molecular mechanism of MSCs in the treatment of IBD, summarize the protocols and improved technical methods currently being developed to enhance the effect of MSCs on the treatment of IBD and provide a basis for more promising and safer prospects for MSC applications.

SEARCH STRATEGY

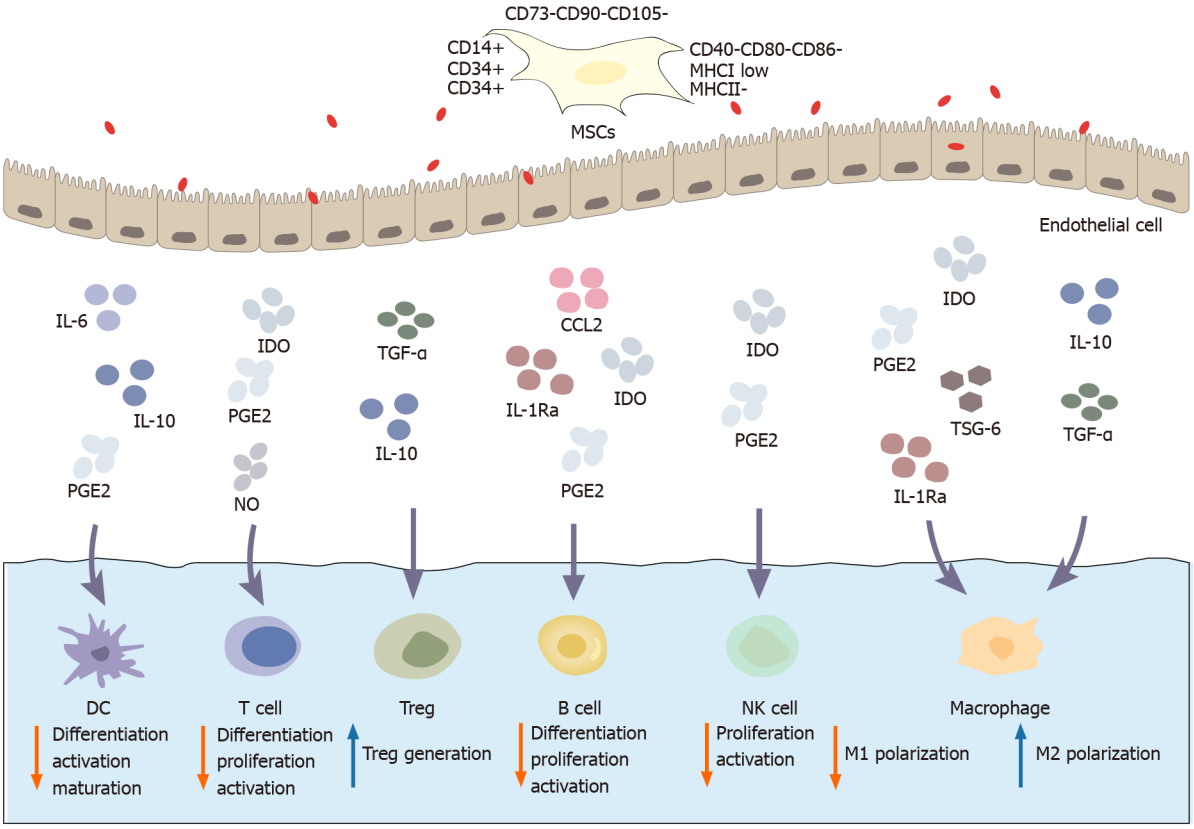
We conducted a comprehensive literature search of the following databases: PubMed, Google Scholar and SpiScholar, using *Reference Citation Analysis* (https://www.referencecitationanalysis.com). Free text words and database-specific index terms were combined with Boolean operators ("AND" and "OR") to improve the sensitivity of our search. We searched for multiple combinations of the following keywords: mesenchymal stem cells, inflammatory bowel disease, hypoxia, inflammatory, pretreatment, preconditioning, stimulation, priming, regeneration, immunomodulation, secretome, conditioned medium (CM), paracrine, therapeutic, brain, nervous system, bone, and cartilage, among others. The identified studies were not constrained by the publication language or publication status. Most of the publications were published in the last five years, but some classical studies are also cited.

USE OF MSCS AND THEIR DERIVATIVES IN THE TREATMENT OF IBD

MSCs are pluripotent stem cells with the ability to self-renew and differentiate into various cell types. These cells play a key role in immune regulation and regenerative therapy[17]. According to the standard definition of MSCs, MSCs express CD73, CD90, CD105 and other markers, but do not express the haematopoietic markers CD45, CD34, CD14, CD19 and HLA-DR (≤ 2%) and differentiate into adipogenic, osteogenic and chondrogenic cells *in vitro*. The sources of MSCs include the bone marrow, fat, muscle, peripheral blood, umbilical cord, placenta, foetal tissue and amniotic fluid[18]. BMMSCs are the most widely used and studied stem cells, but their application is limited due to the possible pain and the incidence rate caused by bone marrow aspiration, and the limited number of BMSCs obtained [19]. Adipose-derived mesenchymal stem cells (AMSCs) and umbilical cord derived mesenchymal stem cells (UCMSCs) have recently received considerable attention. The procedures for collecting UCMSCs are painless, and these cells exhibit faster self-renewal characteristics[20]. Dental mesenchymal stem cells have a strong muscle regeneration ability. After combination with appropriate scaffold materials, these cells provide a favorable alternative treatment for muscle tissue engineering[21]. Human term placental tissue-derived MSCs and the conditioned medium remaining after cultures of these cells reportedly enhance angiogenesis[22]. MSCs derived from the amniotic fluid and amniotic membrane have been introduced as attractive and potent stem cell sources for clinical application due to their easy, safe, and painless collection procedures with minimal ethical issues[23].

MSCs influence the phenotype and function of innate immune cells (macrophages, dendritic cells, neutrophils, eosinophils, basophils, natural killer cells, natural killer T cells, and natural lymphocytes) and acquired immune cells (T and B lymphocytes) through paracrine signalling (secretion of soluble factors) or cell-cell contact[24] (Figure 1). Clinical trials of MSC treatments as a new cell therapy strategy for IBD have yielded certain results, but considerable room for improvement remains. According to a published study, more than 200 patients with refractory fistulas have received local injections of BMMSCs. More than half of the patients achieved complete remission, and approximately two-thirds of the patients achieved overall remission. Among patients with refractory luminal CD, 49 cases of systematic transplantation of MSCs have been recorded, and the results have shown that autologous BMMSCs generate reduced responses, whereas the use of allogeneic BMMSCs is promising because approximately 60% of patients exhibited a response, and approximately 40% achieved clinical remission [25]. In general, bone marrow mesenchymal stem cell transplantation (BMSCT) is presumed to be markedly safer than haematopoietic stem cell transplantation (HSCT). Although autologous BMSCT does not show higher efficacy than conventional treatment, allogeneic BMSCT appeared to be more effective in patients with intracranial CD in a phase II metacentre clinical trial[26]. In addition, clinical research on the treatment of CD patients with AMSCs has been performed. Panés *et al*[27] completed a phase III randomized double-blind trial involving the use of allogeneic AMSC transplantation to treat patients with complex anal fistulas and observed obvious curative effects. These experiments showed the prospects of MSCs in the treatment of IBD.

Although the results of clinical trials are promising in terms of safety and efficacy, many problems remain to be solved, such as the exact working mechanism, dose, mode of administration and optimal concentration. In a trinitrobenzene sulfonic acid (TNBS)-induced colitis model, intravenously injected MSCs were cleared nonspecifically by the innate immune system under physiological conditions. However, local administration may prevent MSCs from directly entering the blood circulation and thereby significantly reduce spleen and liver clearance, and local administration increases the concentration and duration of MSC engraftment in target organs. However, the disadvantage of local administration is that the operation is relatively complex[28]. Similarly, a dextran sulfate sodium (DSS)-induced colitis model was intravenously injected with human embryonic stem cell-derived MSCs (TMSCs), and CM-DiI was used for tracking for 12 and 24 h. The injected TMSCs mainly accumulate in the lung, and a small portion of TMSCs localized to the liver and spleen. However, MSCs expressing fluorescein were only detected at the injection site 12 and 24 h after intrapulmonary administration. No signal was detected after intravenous injection, indicating that most of the intravenously injected TMSCs migrated away from the injection site through the blood circulation, that the number of cells in the target organ



DOI: 10.4252/wjsc.v14.i9.684 **Copyright** ©The Author(s) 2022.

Figure 1 Immunoregulatory mechanism of mesenchymal stem cells. Mesenchymal stem cells (MSCs) express CD73, CD90 and CD105 and do not express haematopoietic markers such as CD14, CD34 and CD45 or the costimulatory molecules CD40, CD80 and CD86. MSCs exhibit low expression of major histocompatibility complex class (MHC) I and do not express MHC II. MSCs possess a wide range of immunomodulatory properties. Activated MSCs secrete a variety of soluble factors, such as indoleamine 2,3-dioxygenase, prostaglandin E2, transforming growth factor-β, tumor necrosis factor-α stimulating gene 6, interleukin (IL)-1Ra, and IL-6. These factors inhibit the differentiation, proliferation and activation of various immune cell subsets, including T cells, B cells, dendritic cells, macrophages, and natural killer cells. Therefore, MSCs inhibit the immune response to inhibit inflammation. MSCs: Mesenchymal stem cells; MHC: Major histocompatibility complex; IDO: Indoleamine 2,3-dioxygenase; PGE2: Prostaglandin E2; TGF-β: Transforming growth factor-β; TSG-6: Tumor necrosis factor-α stimulating gene 6; IL: Interleukin; NK: Natural killer; CCL: CC chemokine ligand.

was too low for bioluminescence imaging, and that the intravenously injected cells may no longer have been viable during the observation period[15]. Despite many unsolved problems, the direct injection of MSCs into target tissues has been indicated to improve the homing efficiency[29]. Arterial MSC infusion is another option, and the major limitation is the risk of intestinal ischaemia due to embolic events[30]. Consequently, MSCs may interact with resident cells by secreting paracrine factors or through intercellular communication[31]. In addition to the low survival rates of stem cells that are injected intravenously into the targeted area to treat intracranial diseases, the injected stem cells may also migrate to other sites and produce side effects. Therefore, some techniques have been developed to promote colonic mucosal healing through interventional radiology and an intra-arterial injection of MSCs into the ileum, and clinical trials have also suggested their safety and efficacy[32]. In a recent study, a temperature-responsive Petri dish was used to endoscopically transplant MSC sheets into the inflammatory area of mice with TNBS-induced colitis. The effect of MSC sheet transplantation on ulcer reduction was then confirmed, verifying that endoscopic MSC transplantation may be a new and effective method for the treatment of IBD[33]. Many inconsistencies regarding the location and persistence of MSCs after transplantation have been reported. Therefore, many recent studies have focused on the paracrine immunomodulatory effects of biological factors secreted by MSCs, particularly the immunomodulatory potential of soluble factors (cytokines, chemokines and growth factors)[31,34]. Cell-free therapy with these derivatives has been proposed as a treatment for IBD. An important advantage of these cell-free therapies is that they may reduce the risk of immune rejection.

MSCs have emerged as a new paradigm for IBD treatment, largely due to their multifaceted biological functions. MSCs secrete numerous factors that target immune cells and affect their functions [35] (Table 1), such as indoleamine 2,3-dioxygenase (IDO), canine urinary quinoline, prostaglandin E2 (PGE2), CD73, transforming growth factor-β (TGF-β), IL-6 and TNF-α stimulating gene 6 (TSG-6), and these effects enable MSCs to regulate T cells, B cells, macrophages, natural killer cells, and dendritic cells [36]. MSCs adjust their immune function in an inflammatory environment, particularly by stimulating the proinflammatory cytokines interferon-γ (IFN-γ) and TNF-α. Activated, MSCs upregulate the

Table 1 Common factors secreted by mesenchymal stem cells[35]

Type	Representative factors
Immunomodulatory factor	HGF, TGF-β1, PGE2, IDO
Chemokine	RANTES, SDF-1α, MIP-1α, MCP-1
Nutritional factors	HGF, NGF, FGF-2, PDGF-AA, PDGF-BB, EGF
Haematopoietic growth factor	G-CSF, M-CSF, GM-CSF, EPO
Vascular regeneration factor	$VEGF_{165}$, FGF-2, EGF, PDGF
Scar inhibiting factor	HGF, FGF-2
Anti-apoptotic factor	$VEGF_{165}$, FGF-2, HGF

HGF: Hepatocyte growth factor; TGF-β: Transforming growth factor-β; PGE2: Prostaglandin E2; IDO: Indoleamine 2,3-dioxygenase; SDF-1α: Stromal cell-derived factor-1α; MCP-1: Monocyte chemoattractant protein-1; NGF: Nerve growth factor; FGF: Fibroblast growth factor; PDGF: Platelet-derived growth factor; G-CSF: Colony stimulating factor 3; M-CSF: Colony stimulating factor 1; GM-CSF: Colony stimulating factor 2; EPO: Erythropoietin; $VEGF_{165}$: Vascular endothelial growth factor-165.

expression of IL-6, IL-10, IDO, TGF, PGE2, hepatocyte growth factor, nitric oxide and haem oxygenase-1 (HO-1)[31].

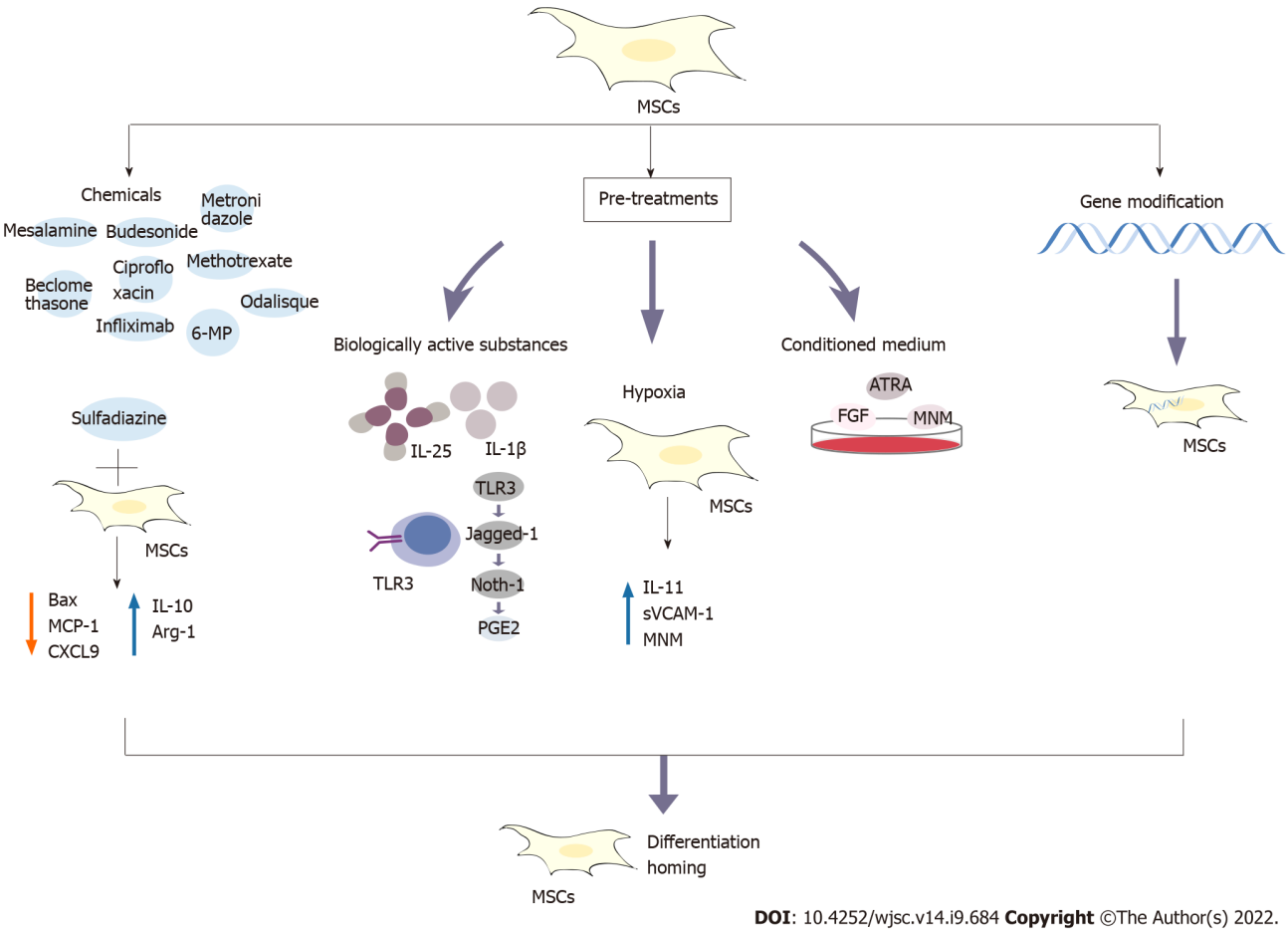
In addition to MSCs, cytokines and extracellular vesicles (EVs) released by MSCs exert therapeutic effects on CD. According to the position statement of the International Society of Exosomes, EVs are particles with a lipid bilayer, that are naturally released from cells and cannot be replicated. MSC EVs perform their functions by transferring their contents, such as proteins, mRNAs and microRNAs (miRNAs), to target cells[37]. EVs have been proven to retain the therapeutic characteristics of MSCs and thereby stimulate tissue repair, limit oxidative stress, reduce inflammation and regulate the immune response. Therefore, an increasing number of studies have focused on the paracrine effects of MSCs[36,38]. In a previous study, researchers compared the effects of MSCs and MSC-derived EVs on IBD in a DSS-induced colitis model and found that MSCs and EVs exert similar immunosuppressive and anti-inflammatory effects by decreasing colonic lymphocyte infiltration and reducing disease severity in DSS-induced mice[39]. Some studies have performed *in situ* injection of EVs to treat colitis. The results show that EV injection regulates the balance of proinflammatory and anti-inflammatory cytokines in colon tissue[40]. As an important paracrine product of MSCs, EVs inhibit the nuclear factor-kappaB (NF-κB) p65 signaling pathway and TNBS-induced colonic oxidative stress in a TNBS-induced experimental colitis model, reduce the production of free radicals, enhance the enzyme defense system, maintain the cellular oxidation/antioxidant balance, and inhibit cell apoptosis through the exogenous death receptor signaling pathway and inherent mitochondrial signaling pathway, which play important roles in colon repair[41]. The experimental data show that EVs promote the M2 polarization of macrophages[42], increase the expression of IL-10, and inhibite IL-1β, TNF-α and IL-6 production. The treatment of RAW264.7 macrophages with EVs upregulates TGF-β1 expression and thereby increases the expression of miR-132. Based on these findings, EVs carry TGF-β1 and regulate the miR-132/mycbp2/TSC2 axis to promote M2 macrophage polarization[43].

ENHANCEMENT OF THE THERAPEUTIC EFFECTS OF MSCS ON IBD

MSCs are typically injected intravenously to treat IBD and may thus remain in blood-rich tissues (liver, lung and spleen) without reaching the target organs. Many animal studies have reported that the level of recruitment and persistence of MSCs *in vivo* is low[44]. Some infusion techniques can be modified or MSCs can be combined with conventional drugs to combat the problems of the low differentiation potential and homing of MSCs to the injured site; additionally, the homing to the target organ and anti-inflammatory effects of MSCs can be enhanced. The enhancement strategies include pretreatment, gene modification and combination with currently used drugs[45]. The pretreatment of MSCs before use comprises a large proportion of improvement strategies. The pretreatment reagents include bioactive substances (cytokines, growth factors and innate immune receptor agonists), hypoxia and modification of the culture medium (Figure 2).

Combination therapy with MSCs and conventional drugs

Currently, patients with IBD are treated with 5-ASA compounds (mainly mesalamine), glucocorticoids (conventional and other forms, such as budesonide and beclomethasone), antibiotics (usually ciprofloxacin and metronidazole), immunomodulators (mainly AZA/6-MP or methotrexate) and biological agents[32,46]. The aim of these treatments is to inhibit intestinal inflammation and ultimately



DOI: 10.4252/wjsc.v14.i9.684 **Copyright** ©The Author(s) 2022.

Figure 2 The developed strategies to improve the efficacy of mesenchymal stem cells in the treatment of inflammatory bowel disease include combined treatment with conventional drugs, pretreatment and gene modification. The tested pretreatments include bioactive factors, hypoxia and medium modification. The conventional drugs include biological preparations of mesalazine, budesonide, beclomethasone, ciprofloxacin, metronidazole, 6-mercaptopurine, methotrexate, infliximab and adalimumab. For example, the combination of mesenchymal stem cells (MSCs) and the drug sulfadiazine inhibits the nuclear factor-kappaB pathway, reduces Bax expression, prevents loss of the B cell lymphoma-2 protein, reduces the levels of monocyte chemoattractant protein-1 and CXCL9, increases the levels of interleukin (IL)-10 and Arg-1, and transforms inflammatory M1 macrophages into anti-inflammatory M2 macrophages. Pretreatment with IL-25 and IL-1β enhances the immunosuppressive abilities of MSCs. MSCs pretreated with Toll-like receptor 3 (TLR3) for a short time *in vitro* produce prostaglandin E2 through the TLR3-Jagged-1-Notch-1 pathway. In response to hypoxia, the levels of IL-11, soluble vascular cell adhesion protein-1 and stromal cell-derived factor-1α are significantly upregulated. MSCs have also been pretreated by modifying the culture medium, such as the addition of fibroblast growth factor, all-trans retinoic acid and modified neuronal medium. In addition, genetically modified MSCs have been developed. These methods and strategies potentially improve the immunosuppressive abilities of MSCs by promoting their homing and differentiation abilities. PGE2: Prostaglandin E2; MSCs: Mesenchymal stem cells; IL: Interleukin; 6-MP: 6-mercaptopurine; MCP-1: Monocyte chemoattractant protein-1; TLR: Toll-like receptor; MNM: Modified neuronal medium; sVCAM-1: Soluble vascular cell adhesion protein-1; ATRA: All-trans retinoic acid; FGF: Fibroblast growth factor.

improve the quality of life of patients with IBD[47]. Biological agents such as infliximab, Odalisque, anti-TNF therapy, α4β7 integrin inhibitors and IL-12/23 inhibitors have changed the treatment of patients with IBD. However, up to 30% of patients with CD and UC do not show a response or do not receive clinical benefits after treatment. In addition, up to half of patients who initially attained clinical benefits lost the second response[48]. In addition to these biological treatments, HSCT has been used to treat some serious gastrointestinal diseases, including IBD[49]. The use of local immunomodulatory cell therapy is an alternative method to the current treatments for IBD. As mentioned above, a phase I-III clinical trial of MSCs to treat IBD has achieved good results, which raises the possibility of combining MSCs with conventional IBD drugs, such as immunosuppressants and anti-inflammatory drugs. However, this strategy remains questionable because whether MSCs and other conventional drugs will affect each other or reduce their functions is unclear. Although some studies suggest that these drugs may affect MSCs, another study showed that MSCs reduce the effects of several immunosuppressive drugs on T-cell subsets in mouse models[50]. A previous study analysed the interaction of immunosuppressive drugs with MSCs in the context of cell proliferation and function[51]. Another study demonstrated that the aggregation of MSCs into globules (when MSCs were injected into a narrow space) resulted in loss of their ability to inhibit T cells. Interestingly, the addition of budesonide to the pellet partially restored the inhibitory effect of MSCs on T-cell proliferation. Although globular MSCs do not inhibit the proliferation of T cells alone, when combined with budesonide, PGE2 produced by

globular MSCs act synergistically with budesonide on EP2-/-EP4 receptors of T cells to inhibit T-cell proliferation[36]. The thiopurine analogues AZA and 6-MP were widely accepted in the early 1980s as agents for the treatment of IBD. The thiopurine metabolite 6-MP is a purine antagonist that inhibits the proliferation of T and B lymphocytes by interfering with DNA and RNA synthesis[47]. The differences in cell polarity and actin organization induced by AZA and dexamethasone (DEX) might reflect the different effects of immunosuppressive drugs on MSC migration. Because the clearance of allogeneic MSCs can be delayed, combined treatment with AZA may improve the homing of BMMSCs to the injured site, to achieve a better therapeutic effect[52]. The combination of AZA and MSCs does not alter their respective effects. In contrast, the combination may improve the therapeutic effects on IBD. A molecular analysis showed that steroids and TNF substantially increase vascular endothelial growth factor C (VEGF-C) production in MSCs, and VEGF-C in turn promote the $CD8^+$ T-cell response to reverse the immunosuppressive effect of MSCs and provide new information for effective MSC therapy [53]. The T-cell subsets, and the Bax (proapoptotic protein) and B cell lymphoma-2 (Bcl-2) (anti-apoptotic protein) levels are unbalanced, which results in defects in immune cell apoptosis in the IBD microenvironment[54]. In a recent study, the NF-κB signaling pathway in the colons of rats with TNBS-induced colitis was effectively inhibited by the combination of AMSCs and sulfadiazine, and the loss of Bcl-2 protein expression was prevented by reduction of Bax expression. A decrease in NF-κB signaling reduces inflammatory, proliferative and proapoptotic activities[55,56]. In addition, the combination of AMSCs and sulfadiazine transforms inflammatory M1 macrophages into anti-inflammatory M2 macrophages by reducing the levels of monocyte chemoattractant protein-1 (MCP-1) and CXCL9 and increasing the levels of IL-10 and Arg-1. Therefore, AMSCs combined with conventional IBD drugs may be a more effective strategy to alleviate the progression of colitis by reducing the levels of inflammatory and apoptosis markers than individual treatments[57]. In addition to routine medication, some relevant studies have examined the efficacy of MSCs combined with other drugs as treatments for IBD. MIS416 is a novel immunomodulatory factor extracted from *Propionibacterium acne* that is composed of MDP and bacterial DNA, and activates the cytoplasmic receptors nucleotide-binding oligomerization domain 2 and Toll-like receptor 9 (TLR9)[58]. The effect of MIS416 on mice with 3% DSS-induced experimental colitis has been analysed. The retro-orbital administration of MIS416 has been followed by subsequent intrapulmonary injection of umbilical cord blood derived MSCs (human UCMSCs). Compared with single administration, the combination of MIS416 with UCMSCs significantly alleviate the symptoms of IBD to improve the treatment efficacy of stem cells. The therapeutic effect is mediated by inhibiting Th1 and Th17 cells, polarization of Th2 cells, and increases in the numbers of Treg and B cells; in particular, the combination of MIS416 and human UCMSCs shifted the balance from Th1/Th17 to a Treg-oriented response. In contrast, improper changes in the immune environment promote increases in the levels of cytokines such as IFN-γ, IL-6, and IL-12, and human UCMSCs are stimulated by these cytokines and subsequently inhibit proinflammatory cells in the inflamed colon. Moreover, MIS416-induced MCP-1 production increases the migration of human UCMSCs, leading to increased colonic infiltration. In conclusion, MIS416 enhances the therapeutic effect of human UCMSCs on experimental colitis by improving immunosuppression and regulating immune homeostasis in the intestine[59]. The combined use of BMMSCs and immunosuppressants may prolong the survival of transplanted BMMSCs and reduce the adverse reactions to drugs to improve the therapeutic effect. Importantly, the use of bioactive reagents to promote homeostasis of the immune balance *in vivo* stabilizes the effects of BMMSCs[54]. A study showed that combined transplantation of MSCs and tryptophan decarboxylases (TDCs) (dendritic cells) is more effective than single therapy in alleviating the clinical and histological manifestations of colitis, particularly compared with MSC transplantation alone. The protective effect of TDC-MSCs is accompanied by the induction of Treg cells and increased production of anti-inflammatory cytokines in the spleen and mesenteric lymph nodes (MLNs). Therefore, the combined transplantation of BMMSCs and TDCs may be a promising and effective method for treating IBD[60]. MSCs combined with new immune agents will be a more effective method for IBD therapy than conventional treatment, and studies exploring agents to enhance the therapeutic effect of MSCs on IBD are urgently needed.

Pretreatment of MSCs

Pretreatment with bioactive substances: Injured or inflamed tissue may release cytokines and growth factors, such as TGF-β1, TNF-α and IL. IL-1β is a member of the IL-1 family and plays a key role in innate immunity and inflammation in a variety of tissues and organs. A previous study showed that IL-1β increases leukocyte migration. Animal experiments have shown that IL-1β increases the migration of BMMSCs to the inflamed spleen, mesentery and colon, repairs the damaged intestinal mucosa and exerts an immunosuppressive effect by increasing the expression of chemokine receptor 3 (CXCR3) and CXCR4[54].

In colitis mice, IL-1β-treated MSCs regulate the balance of immune cells in the spleen and MLNs by increasing the expression of cyclooxygenase-2 (COX-2), IL-6 and IL-8 and altering the polarization of peritoneal macrophages. Importantly, IL-1β-induced MSCs exhibit upregulates CXCR4 expression and better engraftment at the site of intestinal inflammation, which increases the efficacy of IL-1β-induced MSCs in the treatment of DSS-induced colitis[61]. INF-γ induction maintains the classic phenotype of AMSCs without significantly changing the proliferation or migration of AMSCs. However, compared with untreated AMSCs, IFN-γ treated AMSCs produce significantly increased levels of IDO, exhibited

higher expression of adhesion molecule family-1 (ICAM-1), and inhibit the proliferation of activated T cells[62]. Therefore, an experiment using IL-1β and IFN-γ combined with human UCMSCs showed that the pretreated MSCs significantly reduce the proliferation of peripheral blood mononuclear cells, indicating that their immunosuppressive activity is enhanced. Compared with untreated human UCMSCs, PGE2 secretion and the expression of COX-2 and IDO are significantly increased in pretreated human UCMSCs. Thus, DSS-induced colitis might be alleviated by pretreatment with human UCMSCs [63]. In addition, IL-25, which is a member of the IL-17 cytokine family, stimulates the Th2 cell-mediated immune response, and increases the recruitment of inflammatory cells to damaged tissues by affecting epithelial cells[64]. The pretreatment of MSCs with IL-25 may alleviate destructive inflammation in several autoimmune diseases by inhibiting the Th1 or Th17 immune response[65]. Recently, Wang *et al* [66] showed that knocking out IL-25 expression in MSCs eliminates the inhibitory effects of Th17 cells. In addition, MSCs have been manipulated to express CX3CR1 and IL-25 to promote their delivery to the inflamed colon and enhance their immunosuppressive activity[67]. These results help to better clarify the inhibitory potential of AMSCs and their products, and build a foundation for the development of new therapeutic methods to control the immune response. IL-37 exerts a potent immunosuppressive effect on both innate and adaptive immunity. The expression of IL-37 in macrophages or epithelial cells almost completely inhibits the production of proinflammatory cytokines[68]. IL-37-treated MSCs attenuate the histological damage in mice with DSS-induced colitis by inducing the production of Th2-related cytokines and inhibiting splenic production of Th1-related cytokines by $CD4^+$ cells[69]. TLR pathway activation in BMSCs changes their inflammatory characteristics and immunomodulatory effects on cells in the innate and adaptive immune systems[70]. This stimulation of receptors on the cell surface or cytoplasm with corresponding ligands activates the TLR pathway, which involves various adaptor molecules and the transcription factors NF-κB and interferon regulatory factor, resulting in a cytokine response. MSCs express a variety of functional TLRs at high levels, including TLR3 and TLR4, which change the phenotype and immunophenotype of cells after activation[71]. The activation of TLR3 enhances the immunosuppressive activity of MSCs. If human UCMSCs are pretreated with TLR3 for a short time *in vitro*, they produce PGE2 through the TLR3-Jagged-1-Notch-1 pathway and enhance the protective effect of MSCs on TNBS induced colitis in mice[72]. Granulocyte colony stimulating factor (G-CSF) is a glycoprotein that is mainly produced by monocytes and macrophages. G-CSF plays an important role in promoting the differentiation and maturation of haematopoietic cells and the release of mature blood cells[73]. Clinically, G-CSF is mainly used to treat patients with chemotherapy-induced leukopenia and patients with poor responses to peripheral blood stem cell transplantation. The combination of BMSCs and G-CSF in rats increases the number of transplanted MSCs, enhances the immunosuppressive ability of MSCs, inhibits inflammation and reduces leukocyte activation in the intestinal mucosa during UC therapy[74,75]. TSG-6 possesses anti-inflammatory, secretory and tissue-protective properties[76]. The injection of TSG-6 derived from MSC exosomes inhibits the immune response and repairs the damaged tissue, resulting in the alleviation of IBD in mice[77]. In summary, stimulation and pretreatment with these factors may enhance the differentiation and migration of MSCs and exert some immunosuppressive and anti-inflammatory effects. Other researchers have shown that stem cells responding to different types of injury signals will actively secrete endogenous CSF-2, which stimulates MSCs through the PI3K/Akt or FAK/ERK1/2 signaling pathway to increase the differentiation and migration of MSCs. This enhanced therapeutic effect has been proven in an animal model of endometrial ablation[78]. Experiments must be performed to verify whether these factors stimulate MSCs to exert enhanced therapeutic effects and whether they are applicable to IBD. Studies have shown that the intestinal microbiota plays a critical role in IBD[79]. Thus, some experimental models and clinical trials have attempted to correct changes in the gut microbiota (FMT) using various approaches, including microbiota transplantation and probiotic administration[80]. As a treatment for IBD, FMT-MSC transplantation improves the clinical remission rate, enhances the efficacy of radiation therapy against pathogenic bacteria and ultimately restores the intestinal health of patients with IBD[81].

Hypoxic preconditioning of MSCs: MSCs located in inflammatory tissue release many chemokines, which upregulate the expression of cell adhesion molecules, such as ICAM-1 and vascular cell adhesion protein-1 (VCAM-1)[78,82]. These chemokines induce the accumulation of large numbers of CD4+ T cells and Treg cells in the lesion site and enhance the ability of MSCs to regulate the imbalance in immune cells. In recent years, hypoxia has become an effective method to control the proliferation, differentiation and multidirectional differentiation of BMSCs. Hypoxia-treated conditioned medium (HCM) has been proven to exert numerous beneficial effects on tissue regeneration, such as cell recruitment, wound healing, angiogenesis and reconstruction[83]. In long-term culture, the proliferation potential of MSCs cultured under hypoxic conditions is higher than the effect of normobaric oxygen. An oxygen concentration of 1%-5% has been proven to significantly increase the proliferation of MSCs while maintaining their normal morphology[84]. IL-11 is a member of the IL-6 cytokine family and has a structure and function similar to those of IL-6. In recent years, the interest in IL-11 has been renewed due to its unique biological effects on epithelial cancers and inflammatory diseases[85]. IL-11 plays a key role in promoting cell proliferation and protecting cells from oxidative stress[86]. The proteolytic shedding of VCAM-1 also produces soluble VCAM-1 (sVCAM-1), which is present in many cell types of the haematopoietic lineage, including B and T lymphocytes, monocytes, eosinophils and basophils. This

soluble protein plays an important role by mediating leukocyte adhesion and endothelial cell migration during inflammation[87]. Stromal cell-derived factor-1α (SDF1α) is a widely characterized small proinflammatory chemokine that binds to the transmembrane receptor CXCR4[88]. The binding of SDF-1α to CXCR4 induces not only the migration of stem cells but also the expression of adhesion molecules in stem cells. Compared with those in the normobaric oxygen control group, IL-11, the sVCAM-1 and SDF-1α levels are significantly upregulated by hypoxia, and this upregulation increases chemotaxis and reveals their key role in human BMMSC migration and in characterizing the HCM chemotactic components[89,90].

Another experiment showed that BMSCs coated with VCAM1 antibodies (V-MSCs) can be successfully obtained. The analysis showed that V-MSCs and uncoated MSCs had similar surfaces and differentiation potentials. A transwell analysis showed that V-MSCs exhibit higher mobility than uncoated MSCs. The injection of V-MSCs increases the expression of the SRY gene in the diseased colon and resulted in rapid recovery of all disease indices [including weight change, Drug Attitude Inventory (DAI) scores, histological changes and the expression of Ki67 and claudin 1]. The treatment decreases the proportions of proinflammatory Th1 and Th17 cells and increases the proportions of anti-inflammatory Th2 and Treg cells. V-MSCs show enhanced homing and regulate the immune balance in experimental colitis models, which suggests that these cells may be useful in the treatment of IBD or other immune diseases[91]. Similarly, an intravenous injection of MSCs overexpressing ICAM-1 (C3 cells) into mice with DSS-induced IBD decreases the numbers of Th1 and Th17 cells in the spleen while increasing the number of Tregs. A quantitative polymerase chain reaction analysis showed that the infusion of ICAM-1-overexpressing MSCs significantly reduces the IFN-γ and IL-17 mRNA levels and increases the Foxp3 mRNA levels. These cells reduce inflammatory damage by promoting MSC homing to target organs and immune organs to significantly enhance the beneficial effects of MSC treatment[92].

Modification of the culture medium: CM preparations differ from other biological preparations because they represent a mixture of different factors secreted by cells, including growth factors, cytokines, enzymes, nucleic acids and bioactive lipids[93]. Various studies have shown that MSC-derived exosomes or CM exert similar effects on repairing damaged tissues, inhibiting the inflammatory response and regulating the immune response[36]. CM helps to maintain the increased paracrine factor gradient between the diseased organ and the stem cell niche to accelerate the recovery process[94]. Kang *et al*[95] and Wu *et al*[96] showed that using different culture media without foetal bovine serum during the *in vitro* expansion of BMMSCs enhances the immunomodulatory effect of MSCs in an *in vivo* model of IBD. Other researchers then supplemented culture media with different compounds, such as a combination of aspirin, b-fibroblast growth factor (b-FGF), all-trans retinoic acid and modified neuronal medium (MNM) or the combination of activin A, b-FGF and platelet lysates. Studies have been designed to verify whether the therapeutic effect of MSCs *in vivo* could be enhanced through this modification[32, 97], and the results showed that culturing MSCs with specific pretreated culture media increase cell survival, migration, differentiation and secretory functions[98]. Yang *et al*[99] pretreated human UCMSCs with MNM for 24 h, washed off the MNM and replaced the culture medium with minimum essential medium. After two days of growth, the cells were labelled deadapted MSCs (De-hUCMSCs). Compared with human UCMSC treatment, De-hUCMSC treatment resulted in less weight loss in mice with colitis; specifically, this treatment significantly reduces the ulceration, expansion and DAI score of the mice with colitis and significantly decreases crypt damage and inflammatory cell infiltration in the mouse colon. Based on these results, De-hUCMSCs have obvious therapeutic advantages in the treatment of IBD and better improve the symptoms of colitis than unmodified cells.

Genetic modification enhances the therapeutic effects of MSCs

Genetic or particle modification of BMMSCs potentially improves their immunosuppressive abilities [100]. IFN-γ enhances the immunosuppressive characteristics of MSCs[101], and the basic characteristics of MSCs are not changed after the transfection of pcDNA3 carrying 1-IFN-γ. The transfection of MSCs with IFN-γ induces the overexpression of IFN-γ to balance immunity, upregulation of IDO expression and inhibition of the production of cytokines in subjects with intestinal mucositis to ameliorate intestinal inflammation in a DSS-induced colitis model[102]. Based on accumulating evidence, miR-146a is an anti-inflammatory miRNA and a negative regulator of the innate immune response[103]. BMMSCs were transfected with a lentivirus expressing miR-146a in one study. EVs has been isolated from BMMSCs after gene modification and then delivered *via* tail vein injection to the target tissue of TNBS-induced IBD mice. MiR-146a-carrying EVs significantly inhibite the expression of TNF receptor-related factor 6 (TRAF6) and IL-1 receptor-related kinase 1 (IRAK1) in rats with TNBS-induced colitis. Thus, EVs containing miR-146a improve TNBS-induced experimental colitis caused by targeting TRAF6 and IRAK1[104]. 15-LOX-1 is a key regulator of the inflammatory response in the colon and other tissues and is mainly expressed in macrophages[105]. Notably, miR-148b-5p complementarily binds to the 3′ untranslated region of 15-LOX-1 mRNA, and human UCMSCs transfected with miR148b-5p relieve IBD by downregulating the expression of 15-LOX-1 in macrophages[106]. SDF-1 has been recognized as one of the most critical factors for stem cell homing to the bone marrow and other damaged tissues[107]. The CXCR4 gene has been transfected into BMMSCs with a lentiviral vector, and overexpression of the

CXCR4 gene does not alter the biological characteristics or viability of BMMSCs but increases the migration and homing of BMMSCs *in vitro* and *in vivo*. The overexpression of CXCR4 may promote the homing of BMMSCs to the damaged intestinal mucosa and improve its therapeutic effect on colitis[108]. Overexpression of CXCR4 also exerts a more obvious antitumor effect, which is helpful for preventing and treating the most serious complications of IBD, such as colitis-associated cancer[109]. COX2 is an enzyme involved in arachidonic acid metabolism that is responsible for the production of PGE2. This major inflammatory regulator maintains the immune balance and has been proven to play a vital role in the treatment of IBD with MSCs[110]. COX2 increases the expression of insulin-like growth factor-1 (IGF-1) in the skeletal muscle of MSC-transplanted mice[111,112]. CXCR3 is a G-protein-coupled seven transmembrane receptor that is expressed in damaged parenchymal cells in lesions of multiple organs and inflammatory cells, including activated lymphocytes, macrophages and dendritic cells[113]. HO-1 and its metabolites exert antioxidant, anti-inflammatory, antiproliferative and immunomodulatory effects[114]. The transfection of HO-1 improves the transformation and antioxidant capacity of BMMSCs. A study also showed that the number of BMMSCs modified by CXCR3 and HO-1 are significantly increased at the site of injury and that the damage to intestinal function exhibited rapid recovery. BMMSCs modified with the CXCR3 and/or HO-1 genes were transplanted into the intestinal epithelial recess cell line-6 injury model, and CXCR3 overexpression improves BMMSC chemotaxis to induce the early and rapid recruitment of BMMSCs to damaged intestinal epithelial cells[115,116]. Other experiments have shown that the exogenous or endogenous overexpression of heparin binding epidermal growth factor-like growth factor (HBEGF) promotes the proliferation and migration of MSCs, and the synergistic effect of HBEGF and MSCs further restores the function of the intestinal barrier[117, 118]. The transfection of MSCs with an IL-33 overexpression plasmid reduces apoptosis in early MSCs and further improves the therapeutic effects of MSCs on myocardial infarction (MI) through the polarization of macrophages and T cells[119]. Similarly, in an animal model of MI, BMMSCs overexpressing VEGF and Bcl-2, which inhibit apoptosis and autophagy and enhance paracrine signaling, significantly improves cardiac function by improving the survival rate and angiogenesis, and these modified MSCs are more resistant to harsh environments than unmodified cells[120]. In addition, pioglitazone combined with BMMSC transplantation further enhances the protective effect of BMMSCs on diabetes and heart damage[121]. These studies showed the improved abilities of MSCs to repair damaged tissues, but unfortunately, a study of IBD therapy has not been performed. Research on MSC gene editing in other disease models also provides some insights into the future treatment of IBD with MSCs.

CONCLUSION

MSCs exist widely in human tissues and organs. They not only exhibit multidirectional differentiation and proliferation potential but also show a wide range of prospects and applications in medicine because of their immunoregulatory activity. MSCs represent a safe and effective treatment for IBD. Although MSC therapy has shown great potential in a large number of animal experiments and clinical trials, a consensus on the effectiveness of intravenous MSCs in IBD-focused clinical trials has not been achieved. Many animal studies have suggested that the level of recruitment and persistence of mesenchymal stem cells *in vivo* is low, and thus we must improve strategies to enhance their therapeutic effects. We summarized the strategies to improve the efficacy of MSCs in the treatment of IBD. MSCs have been combined with current drugs, cultured in different media or pretreated with cytokines and biological factors, combined with current IBD treatments and subjected to genetic engineering. These new strategies may increase the efficacy of MSC-based treatments, and further research is ongoing. The goal of IBD treatment remains the same: To achieve prolonged remission and halt any ongoing disease progression. In future research, we must strive to improve the safety and feasibility of MSCs in IBD therapy, improve patient quality of life and maximize the utilization of MSCs.

FOOTNOTES

Author contributions: Shi MY collected the literature and wrote the manuscript; Liu L revised the manuscript; Yang FY designed, wrote, edited and prepared the manuscript for submission; and all authors read and approved the final manuscript.

Supported by the Science and Technology Research Project of Hubei Province, No. 2021CFB210.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country/Territory of origin: China

ORCID number: Meng-Yue Shi 0000-0002-3890-8335; Fu-Yuan Yang 0000-0001-9179-195X.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

REFERENCES

1 **Ramos GP**, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. *Mayo Clin Proc* 2019; **94**: 155-165 [PMID: 30611442 DOI: 10.1016/j.mayocp.2018.09.013]
2 **Pei XF**, Cao LL, Huang F, Qiao X, Yu J, Ye H, Xi CL, Zhou QC, Zhang GF, Gong ZL. Role of miR-22 in intestinal mucosa tissues and peripheral blood CD4+ T cells of inflammatory bowel disease. *Pathol Res Pract* 2018; **214**: 1095-1104 [PMID: 29880327 DOI: 10.1016/j.prp.2018.04.009]
3 **GBD 2017 Inflammatory Bowel Disease Collaborators**. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2020; **5**: 17-30 [PMID: 31648971 DOI: 10.1016/S2468-1253(19)30333-4]
4 **Cui G**, Yuan A. A Systematic Review of Epidemiology and Risk Factors Associated With Chinese Inflammatory Bowel Disease. *Front Med (Lausanne)* 2018; **5**: 183 [PMID: 29971235 DOI: 10.3389/fmed.2018.00183]
5 **Mak WY**, Zhao M, Ng SC, Burisch J. The epidemiology of inflammatory bowel disease: East meets west. *J Gastroenterol Hepatol* 2020; **35**: 380-389 [PMID: 31596960 DOI: 10.1111/jgh.14872]
6 **Rogler G**, Singh A, Kavanaugh A, Rubin DT. Extraintestinal Manifestations of Inflammatory Bowel Disease: Current Concepts, Treatment, and Implications for Disease Management. *Gastroenterology* 2021; **161**: 1118-1132 [PMID: 34358489 DOI: 10.1053/j.gastro.2021.07.042]
7 **Argollo M**, Gilardi D, Peyrin-Biroulet C, Chabot JF, Peyrin-Biroulet L, Danese S. Comorbidities in inflammatory bowel disease: a call for action. *Lancet Gastroenterol Hepatol* 2019; **4**: 643-654 [PMID: 31171484 DOI: 10.1016/S2468-1253(19)30173-6]
8 **Iskandar HN**, Dhere T, Farraye FA. Ulcerative Colitis: Update on Medical Management. *Curr Gastroenterol Rep* 2015; **17**: 44 [PMID: 26386686 DOI: 10.1007/s11894-015-0466-9]
9 **Misselwitz B**, Juillerat P, Sulz MC, Siegmund B, Brand S; Swiss IBDnet, an official working group of the Swiss Society of Gastroenterology. Emerging Treatment Options in Inflammatory Bowel Disease: Janus Kinases, Stem Cells, and More. *Digestion* 2020; **101** Suppl 1: 69-82 [PMID: 32570252 DOI: 10.1159/000507782]
10 **Troncone E**, Monteleone G. The safety of non-biological treatments in Ulcerative Colitis. *Expert Opin Drug Saf* 2017; **16**: 779-789 [PMID: 28608717 DOI: 10.1080/14740338.2017.1340936]
11 **Shi X**, Chen Q, Wang F. Mesenchymal stem cells for the treatment of ulcerative colitis: a systematic review and meta-analysis of experimental and clinical studies. *Stem Cell Res Ther* 2019; **10**: 266 [PMID: 31443677 DOI: 10.1186/s13287-019-1336-4]
12 **Joustra V**, Duijvestein M, Mookhoek A, Bemelman W, Buskens C, Koželj M, Novak G, Hindryckx P, Mostafavi N, D'Haens G. Natural History and Risk Stratification of Recurrent Crohn's Disease After Ileocolonic Resection: A Multicenter Retrospective Cohort Study. *Inflamm Bowel Dis* 2022; **28**: 1-8 [PMID: 33783507 DOI: 10.1093/ibd/izab044]
13 **Lightner AL**, Faubion WA. Mesenchymal Stem Cell Injections for the Treatment of Perianal Crohn's Disease: What We Have Accomplished and What We Still Need to Do. *J Crohns Colitis* 2017; **11**: 1267-1276 [PMID: 28387832 DOI: 10.1093/ecco-jcc/jjx046]
14 **Nagaishi K**, Arimura Y, Fujimiya M. Stem cell therapy for inflammatory bowel disease. *J Gastroenterol* 2015; **50**: 280-286 [PMID: 25618180 DOI: 10.1007/s00535-015-1040-9]
15 **Xu J**, Wang X, Chen J, Chen S, Li Z, Liu H, Bai Y, Zhi F. Embryonic stem cell-derived mesenchymal stem cells promote colon epithelial integrity and regeneration by elevating circulating IGF-1 in colitis mice. *Theranostics* 2020; **10**: 12204-12222 [PMID: 33204338 DOI: 10.7150/thno.47683]
16 **Shi Y**, Wang Y, Li Q, Liu K, Hou J, Shao C. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. *Nat Rev Nephrol* 2018; **14**: 493-507 [PMID: 29895977 DOI: 10.1038/s41581-018-0023-5]
17 **Jasim SA**, Yumashev AV, Abdelbasset WK, Margiana R, Markov A, Suksatan W, Pineda B, Thangavelu L, Ahmadi SH. Shining the light on clinical application of mesenchymal stem cell therapy in autoimmune diseases. *Stem Cell Res Ther* 2022; **13**: 101 [PMID: 35255979 DOI: 10.1186/s13287-022-02782-7]
18 **Brown C**, McKee C, Bakshi S, Walker K, Hakman E, Halassy S, Svinarich D, Dodds R, Govind CK, Chaudhry GR. Mesenchymal stem cells: Cell therapy and regeneration potential. *J Tissue Eng Regen Med* 2019; **13**: 1738-1755 [PMID: 31216380 DOI: 10.1002/term.2914]
19 **Mohamed-Ahmed S**, Fristad I, Lie SA, Suliman S, Mustafa K, Vindenes H, Idris SB. Adipose-derived and bone marrow mesenchymal stem cells: a donor-matched comparison. *Stem Cell Res Ther* 2018; **9**: 168 [PMID: 29921311 DOI: 10.1186/s13287-018-0914-1]
20 **Ding DC**, Chang YH, Shyu WC, Lin SZ. Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy. *Cell Transplant* 2015; **24**: 339-347 [PMID: 25622293 DOI: 10.3727/096368915X686841]
21 **Ansari S**, Chen C, Xu X, Annabi N, Zadeh HH, Wu BM, Khademhosseini A, Shi S, Moshaverinia A. Muscle Tissue

Engineering Using Gingival Mesenchymal Stem Cells Encapsulated in Alginate Hydrogels Containing Multiple Growth Factors. *Ann Biomed Eng* 2016; **44**: 1908-1920 [PMID: 27009085 DOI: 10.1007/s10439-016-1594-6]

22 **Komaki M**, Numata Y, Morioka C, Honda I, Tooi M, Yokoyama N, Ayame H, Iwasaki K, Taki A, Oshima N, Morita I. Exosomes of human placenta-derived mesenchymal stem cells stimulate angiogenesis. *Stem Cell Res Ther* 2017; **8**: 219 [PMID: 28974256 DOI: 10.1186/s13287-017-0660-9]

23 **Testrow S**, McGovern R, Tully V. Secondary care interface: optimising communication between teams within secondary care to improve the rehabilitation journey for older people. *BMJ Open Qual* 2021; **10** [PMID: 33579746 DOI: 10.1136/bmjoq-2020-001274]

24 **Harrell CR**, Volarevic V. Apoptosis: A friend or foe in mesenchymal stem cell-based immunosuppression. *Adv Protein Chem Struct Biol* 2021; **126**: 39-62 [PMID: 34090619 DOI: 10.1016/bs.apcsb.2021.01.002]

25 **Grégoire C**, Lechanteur C, Briquet A, Baudoux É, Baron F, Louis E, Beguin Y. Review article: mesenchymal stromal cell therapy for inflammatory bowel diseases. *Aliment Pharmacol Ther* 2017; **45**: 205-221 [PMID: 27878827 DOI: 10.1111/apt.13864]

26 **Forbes GM**, Sturm MJ, Leong RW, Sparrow MP, Segarajasingam D, Cummins AG, Phillips M, Herrmann RP. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. *Clin Gastroenterol Hepatol* 2014; **12**: 64-71 [PMID: 23872668 DOI: 10.1016/j.cgh.2013.06.021]

27 **Panés J**, García-Olmo D, Van Assche G, Colombel JF, Reinisch W, Baumgart DC, Dignass A, Nachury M, Ferrante M, Kazemi-Shirazi L, Grimaud JC, de la Portilla F, Goldin E, Richard MP, Leselbaum A, Danese S; ADMIRE CD Study Group Collaborators. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. *Lancet* 2016; **388**: 1281-1290 [PMID: 27477896 DOI: 10.1016/S0140-6736(16)31203-X]

28 **Cao X**, Duan L, Hou H, Liu Y, Chen S, Zhang S, Wang C, Qi X, Liu N, Han Z, Zhang D, Han ZC, Guo Z, Zhao Q, Li Z. IGF-1C hydrogel improves the therapeutic effects of MSCs on colitis in mice through PGE_2-mediated M2 macrophage polarization. *Theranostics* 2020; **10**: 7697-7709 [PMID: 32685014 DOI: 10.7150/thno.45434]

29 **Liesveld JL**, Sharma N, Aljitawi OS. Stem cell homing: From physiology to therapeutics. *Stem Cells* 2020; **38**: 1241-1253 [PMID: 32526037 DOI: 10.1002/stem.3242]

30 **Lightner AL**. Stem Cell Therapies for Inflammatory Bowel Disease. *Curr Gastroenterol Rep* 2019; **21**: 16 [PMID: 30955111 DOI: 10.1007/s11894-019-0672-y]

31 **da Costa Gonçalves F**, Paz AH. Cell membrane and bioactive factors derived from mesenchymal stromal cells: Cell-free based therapy for inflammatory bowel diseases. *World J Stem Cells* 2019; **11**: 618-633 [PMID: 31616539 DOI: 10.4252/wjsc.v11.i9.618]

32 **Lopez-Santalla M**, Garin MI. Improving the Efficacy of Mesenchymal Stem/Stromal-Based Therapy for Treatment of Inflammatory Bowel Diseases. *Biomedicines* 2021; **9** [PMID: 34829736 DOI: 10.3390/biomedicines9111507]

33 **Pak S**, Hwang SW, Shim IK, Bae SM, Ryu YM, Kim HB, Do EJ, Son HN, Choi EJ, Park SH, Kim SY, Ye BD, Yang SK, Kanai N, Maeda M, Okano T, Yang DH, Byeon JS, Myung SJ. Endoscopic Transplantation of Mesenchymal Stem Cell Sheets in Experimental Colitis in Rats. *Sci Rep* 2018; **8**: 11314 [PMID: 30054522 DOI: 10.1038/s41598-018-29617-x]

34 **Crivelli B**, Chlapanidas T, Perteghella S, Lucarelli E, Pascucci L, Brini AT, Ferrero I, Marazzi M, Pessina A, Torre ML; Italian Mesenchymal Stem Cell Group (GISM). Mesenchymal stem/stromal cell extracellular vesicles: From active principle to next generation drug delivery system. *J Control Release* 2017; **262**: 104-117 [PMID: 28736264 DOI: 10.1016/j.jconrel.2017.07.023]

35 **Samsonraj RM**, Rai B, Sathiyanathan P, Puan KJ, Rötzschke O, Hui JH, Raghunath M, Stanton LW, Nurcombe V, Cool SM. Establishing criteria for human mesenchymal stem cell potency. *Stem Cells* 2015; **33**: 1878-1891 [PMID: 25752682 DOI: 10.1002/stem.1982]

36 **Burand AJ Jr**, Di L, Boland LK, Boyt DT, Schrodt MV, Santillan DA, Ankrum JA. Aggregation of Human Mesenchymal Stromal Cells Eliminates Their Ability to Suppress Human T Cells. *Front Immunol* 2020; **11**: 143 [PMID: 32158443 DOI: 10.3389/fimmu.2020.00143]

37 **Qiu G**, Zheng G, Ge M, Wang J, Huang R, Shu Q, Xu J. Functional proteins of mesenchymal stem cell-derived extracellular vesicles. *Stem Cell Res Ther* 2019; **10**: 359 [PMID: 31779700 DOI: 10.1186/s13287-019-1484-6]

38 **Gonzalez-Pujana A**, Igartua M, Santos-Vizcaino E, Hernandez RM. Mesenchymal stromal cell based therapies for the treatment of immune disorders: recent milestones and future challenges. *Expert Opin Drug Deliv* 2020; **17**: 189-200 [PMID: 31918562 DOI: 10.1080/17425247.2020.1714587]

39 **Li Y**, Altemus J, Lightner AL. Mesenchymal stem cells and acellular products attenuate murine induced colitis. *Stem Cell Res Ther* 2020; **11**: 515 [PMID: 33256827 DOI: 10.1186/s13287-020-02025-7]

40 **Duan L**, Huang H, Zhao X, Zhou M, Chen S, Wang C, Han Z, Han ZC, Guo Z, Li Z, Cao X. Extracellular vesicles derived from human placental mesenchymal stem cells alleviate experimental colitis in mice by inhibiting inflammation and oxidative stress. *Int J Mol Med* 2020; **46**: 1551-1561 [PMID: 32945344 DOI: 10.3892/ijmm.2020.4679]

41 **Yang J**, Liu XX, Fan H, Tang Q, Shou ZX, Zuo DM, Zou Z, Xu M, Chen QY, Peng Y, Deng SJ, Liu YJ. Extracellular Vesicles Derived from Bone Marrow Mesenchymal Stem Cells Protect against Experimental Colitis *via* Attenuating Colon Inflammation, Oxidative Stress and Apoptosis. *PLoS One* 2015; **10**: e0140551 [PMID: 26469068 DOI: 10.1371/journal.pone.0140551]

42 **Harrell CR**, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Mesenchymal Stem Cell-Derived Exosomes and Other Extracellular Vesicles as New Remedies in the Therapy of Inflammatory Diseases. *Cells* 2019; **8** [PMID: 31835680 DOI: 10.3390/cells8121605]

43 **Wang Y**, Han B, Wang Y, Wang C, Zhang H, Xue J, Wang X, Niu T, Niu Z, Chen Y. Mesenchymal stem cell-secreted extracellular vesicles carrying TGF-β1 up-regulate miR-132 and promote mouse M2 macrophage polarization. *J Cell Mol Med* 2020; **24**: 12750-12764 [PMID: 32965772 DOI: 10.1111/jcmm.15860]

44 **Guo YC**, Chiu YH, Chen CP, Wang HS. Interleukin-1β induces CXCR3-mediated chemotaxis to promote umbilical cord mesenchymal stem cell transendothelial migration. *Stem Cell Res Ther* 2018; **9**: 281 [PMID: 30359318 DOI: 10.1186/s13287-018-1032-9]

45 **Hu C**, Wu Z, Li L. Pre-treatments enhance the therapeutic effects of mesenchymal stem cells in liver diseases. *J Cell Mol Med* 2020; **24**: 40-49 [PMID: 31691463 DOI: 10.1111/jcmm.14788]

46 **Magro F**, Cordeiro G, Dias AM, Estevinho MM. Inflammatory Bowel Disease - Non-biological treatment. *Pharmacol Res* 2020; **160**: 105075 [PMID: 32653651 DOI: 10.1016/j.phrs.2020.105075]

47 **Grevenitis P**, Thomas A, Lodhia N. Medical Therapy for Inflammatory Bowel Disease. *Surg Clin North Am* 2015; **95**: 1159-1182, vi [PMID: 26596920 DOI: 10.1016/j.suc.2015.08.004]

48 **Papamichael K**, Cheifetz AS. Therapeutic drug monitoring in inflammatory bowel disease: for every patient and every drug? *Curr Opin Gastroenterol* 2019; **35**: 302-310 [PMID: 30973355 DOI: 10.1097/MOG.0000000000000536]

49 **Cassinotti A**, Passamonti F, Segato S. CELL THERAPY IN INFLAMMATORY BOWEL DISEASE. *Pharmacol Res* 2021; **163**: 105247 [PMID: 33069755 DOI: 10.1016/j.phrs.2020.105247]

50 **Hajkova M**, Hermankova B, Javorkova E, Bohacova P, Zajicova A, Holan V, Krulova M. Mesenchymal Stem Cells Attenuate the Adverse Effects of Immunosuppressive Drugs on Distinct T Cell Subopulations. *Stem Cell Rev Rep* 2017; **13**: 104-115 [PMID: 27866327 DOI: 10.1007/s12015-016-9703-3]

51 **Javorkova E**, Vackova J, Hajkova M, Hermankova B, Zajicova A, Holan V, Krulova M. The effect of clinically relevant doses of immunosuppressive drugs on human mesenchymal stem cells. *Biomed Pharmacother* 2018; **97**: 402-411 [PMID: 29091890 DOI: 10.1016/j.biopha.2017.10.114]

52 **Schneider N**, Gonçalves Fda C, Pinto FO, Lopez PL, Araújo AB, Pfaffenseller B, Passos EP, Cirne-Lima EO, Meurer L, Lamers ML, Paz AH. Dexamethasone and azathioprine promote cytoskeletal changes and affect mesenchymal stem cell migratory behavior. *PLoS One* 2015; **10**: e0120538 [PMID: 25756665 DOI: 10.1371/journal.pone.0120538]

53 **Gan Y**, Zhang T, Chen X, Cao W, Lin L, Du L, Wang Y, Zhou F, He X, He Y, Gan J, Sheng H, Sorokin L, Shi Y. Steroids Enable Mesenchymal Stromal Cells to Promote $CD8^+$ T Cell Proliferation Via VEGF-C. *Adv Sci (Weinh)* 2021; **8**: 2003712 [PMID: 34194927 DOI: 10.1002/advs.202003712]

54 **Lee BC**, Kang KS. Functional enhancement strategies for immunomodulation of mesenchymal stem cells and their therapeutic application. *Stem Cell Res Ther* 2020; **11**: 397 [PMID: 32928306 DOI: 10.1186/s13287-020-01920-3]

55 **Hamza AA**, Lashin FM, Gamel M, Hassanin SO, Abdalla Y, Amin A. Hawthorn Herbal Preparation from *Crataegus oxyacantha* Attenuates In Vivo Carbon Tetrachloride -Induced Hepatic Fibrosis *via* Modulating Oxidative Stress and Inflammation. *Antioxidants (Basel)* 2020; **9** [PMID: 33255507 DOI: 10.3390/antiox9121173]

56 **Hamza AA**, Heeba GH, Hamza S, Abdalla A, Amin A. Standardized extract of ginger ameliorates liver cancer by reducing proliferation and inducing apoptosis through inhibition oxidative stress/ inflammation pathway. *Biomed Pharmacother* 2021; **134**: 111102 [PMID: 33338743 DOI: 10.1016/j.biopha.2020.111102]

57 **Yousefi-Ahmadipour A**, Rashidian A, Mirzaei MR, Farsinejad A, PourMohammadi-Nejad F, Ghazi-Khansari M, Ai J, Shirian S, Allahverdi A, Saremi J, Ebrahimi-Barough S. Combination therapy of mesenchymal stromal cells and sulfasalazine attenuates trinitrobenzene sulfonic acid induced colitis in the rat: The S1P pathway. *J Cell Physiol* 2019; **234**: 11078-11091 [PMID: 30580446 DOI: 10.1002/jcp.27944]

58 **Mainini F**, Larsen DS, Webster GA, Young SL, Eccles MR. MIS416 as a siRNA Delivery System with the Ability to Target Antigen-Presenting Cells. *Nucleic Acid Ther* 2018; **28**: 225-232 [PMID: 29893623 DOI: 10.1089/nat.2017.0695]

59 **Lee BC**, Shin N, Lee JY, Kang I, Kim JJ, Lee SE, Choi SW, Webster GA, Kang KS. MIS416 Enhances Therapeutic Functions of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells Against Experimental Colitis by Modulating Systemic Immune Milieu. *Front Immunol* 2018; **9**: 1078 [PMID: 29892282 DOI: 10.3389/fimmu.2018.01078]

60 **Abbasi-Kenarsari H**, Heidari N, Baghaei K, Amani D, Zali MR, Gaffari Khaligh S, Shafiee A, Hashemi SM. Synergistic therapeutic effect of mesenchymal stem cells and tolerogenic dendritic cells in an acute colitis mouse model. *Int Immunopharmacol* 2020; **88**: 107006 [PMID: 33182049 DOI: 10.1016/j.intimp.2020.107006]

61 **Fan H**, Zhao G, Liu L, Liu F, Gong W, Liu X, Yang L, Wang J, Hou Y. Pre-treatment with IL-1β enhances the efficacy of MSC transplantation in DSS-induced colitis. *Cell Mol Immunol* 2012; **9**: 473-481 [PMID: 23085948 DOI: 10.1038/cmi.2012.40]

62 **Serejo TRT**, Silva-Carvalho AÉ, Braga LDCF, Neves FAR, Pereira RW, Carvalho JL, Saldanha-Araujo F. Assessment of the Immunosuppressive Potential of INF-γ Licensed Adipose Mesenchymal Stem Cells, Their Secretome and Extracellular Vesicles. *Cells* 2019; **8** [PMID: 30621275 DOI: 10.3390/cells8010022]

63 **Yu Y**, Yoo SM, Park HH, Baek SY, Kim YJ, Lee S, Kim YL, Seo KW, Kang KS. Preconditioning with interleukin-1 beta and interferon-gamma enhances the efficacy of human umbilical cord blood-derived mesenchymal stem cells-based therapy *via* enhancing prostaglandin E2 secretion and indoleamine 2,3-dioxygenase activity in dextran sulfate sodium-induced colitis. *J Tissue Eng Regen Med* 2019; **13**: 1792-1804 [PMID: 31293088 DOI: 10.1002/term.2930]

64 **Bredo G**, Storie J, Shrestha Palikhe N, Davidson C, Adams A, Vliagoftis H, Cameron L. Interleukin-25 initiates Th2 differentiation of human CD4(+) T cells and influences expression of its own receptor. *Immun Inflamm Dis* 2015; **3**: 455-468 [PMID: 26734466 DOI: 10.1002/iid3.87]

65 **Su J**, Xie C, Fan Y, Cheng W, Hu Y, Huang Q, Shi H, Wang L, Ren J. Interleukin-25 enhances the capacity of mesenchymal stem cells to induce intestinal epithelial cell regeneration. *Am J Transl Res* 2017; **9**: 5320-5331 [PMID: 29312486]

66 **Wang WB**, Yen ML, Liu KJ, Hsu PJ, Lin MH, Chen PM, Sudhir PR, Chen CH, Sytwu HK, Yen BL. Interleukin-25 Mediates Transcriptional Control of PD-L1 *via* STAT3 in Multipotent Human Mesenchymal Stromal Cells (hMSCs) to Suppress Th17 Responses. *Stem Cell Reports* 2015; **5**: 392-404 [PMID: 26321145 DOI: 10.1016/j.stemcr.2015.07.013]

67 **Fu Y**, Ni J, Chen J, Ma G, Zhao M, Zhu S, Shi T, Zhu J, Huang Z, Zhang J. Dual-Functionalized MSCs that Express CX3CR1 and IL-25 Exhibit Enhanced Therapeutic Effects on Inflammatory Bowel Disease. *Mol Ther* 2020; **28**: 1214-1228 [PMID: 32087149 DOI: 10.1016/j.ymthe.2020.01.020]

68 **Su Z**, Tao X. Current Understanding of IL-37 in Human Health and Disease. *Front Immunol* 2021; **12**: 696605 [PMID: 34248996 DOI: 10.3389/fimmu.2021.696605]

69 **Wang WQ**, Dong K, Zhou L, Jiao GH, Zhu CZ, Li WW, Yu G, Wu WT, Chen S, Sun ZN, Wang YM, Liu WT, Zhang J, Wang BM, Feng XM. IL-37b gene transfer enhances the therapeutic efficacy of mesenchumal stromal cells in DSS-

induced colitis mice. *Acta Pharmacol Sin* 2015; **36**: 1377-1387 [PMID: 26190499 DOI: 10.1038/aps.2015.51]
70 **Kordjazy N**, Haj-Mirzaian A, Rohani MM, Gelfand EW, Rezaei N, Abdolghaffari AH. Role of toll-like receptors in inflammatory bowel disease. *Pharmacol Res* 2018; **129**: 204-215 [PMID: 29155256 DOI: 10.1016/j.phrs.2017.11.017]
71 **Abdi J**, Rashedi I, Keating A. Concise Review: TLR Pathway-miRNA Interplay in Mesenchymal Stromal Cells: Regulatory Roles and Therapeutic Directions. *Stem Cells* 2018; **36**: 1655-1662 [PMID: 30171669 DOI: 10.1002/stem.2902]
72 **Qiu Y**, Guo J, Mao R, Chao K, Chen BL, He Y, Zeng ZR, Zhang SH, Chen MH. TLR3 preconditioning enhances the therapeutic efficacy of umbilical cord mesenchymal stem cells in TNBS-induced colitis *via* the TLR3-Jagged-1-Notch-1 pathway. *Mucosal Immunol* 2017; **10**: 727-742 [PMID: 27649928 DOI: 10.1038/mi.2016.78]
73 **Lotfi N**, Thome R, Rezaei N, Zhang GX, Rezaei A, Rostami A, Esmaeil N. Roles of GM-CSF in the Pathogenesis of Autoimmune Diseases: An Update. *Front Immunol* 2019; **10**: 1265 [PMID: 31275302 DOI: 10.3389/fimmu.2019.01265]
74 **Tang Y**, Chen Y, Wang X, Song G, Li Y, Shi L. Combinatorial Intervention with Mesenchymal Stem Cells and Granulocyte Colony-Stimulating Factor in a Rat Model of Ulcerative Colitis. *Dig Dis Sci* 2015; **60**: 1948-1957 [PMID: 25894931 DOI: 10.1007/s10620-015-3655-3]
75 **Castro-Dopico T**, Fleming A, Dennison TW, Ferdinand JR, Harcourt K, Stewart BJ, Cader Z, Tuong ZK, Jing C, Lok LSC, Mathews RJ, Portet A, Kaser A, Clare S, Clatworthy MR. GM-CSF Calibrates Macrophage Defense and Wound Healing Programs during Intestinal Infection and Inflammation. *Cell Rep* 2020; **32**: 107857 [PMID: 32640223 DOI: 10.1016/j.celrep.2020.107857]
76 **Day AJ**, Milner CM. TSG-6: A multifunctional protein with anti-inflammatory and tissue-protective properties. *Matrix Biol* 2019; **78-79**: 60-83 [PMID: 29362135 DOI: 10.1016/j.matbio.2018.01.011]
77 **Yang S**, Liang X, Song J, Li C, Liu A, Luo Y, Ma H, Tan Y, Zhang X. A novel therapeutic approach for inflammatory bowel disease by exosomes derived from human umbilical cord mesenchymal stem cells to repair intestinal barrier *via* TSG-6. *Stem Cell Res Ther* 2021; **12**: 315 [PMID: 34051868 DOI: 10.1186/s13287-021-02404-8]
78 **Park SR**, Cho A, Kim JW, Lee HY, Hong IS. A Novel Endogenous Damage Signal, CSF-2, Activates Multiple Beneficial Functions of Adipose Tissue-Derived Mesenchymal Stem Cells. *Mol Ther* 2019; **27**: 1087-1100 [PMID: 30962162 DOI: 10.1016/j.ymthe.2019.03.010]
79 **Weingarden AR**, Vaughn BP. Intestinal microbiota, fecal microbiota transplantation, and inflammatory bowel disease. *Gut Microbes* 2017; **8**: 238-252 [PMID: 28609251 DOI: 10.1080/19490976.2017.1290757]
80 **Aggeletopoulou I**, Konstantakis C, Assimakopoulos SF, Triantos C. The role of the gut microbiota in the treatment of inflammatory bowel diseases. *Microb Pathog* 2019; **137**: 103774 [PMID: 31586663 DOI: 10.1016/j.micpath.2019.103774]
81 **Ocansey DKW**, Wang L, Wang J, Yan Y, Qian H, Zhang X, Xu W, Mao F. Mesenchymal stem cell-gut microbiota interaction in the repair of inflammatory bowel disease: an enhanced therapeutic effect. *Clin Transl Med* 2019; **8**: 31 [PMID: 31872304 DOI: 10.1186/s40169-019-0251-8]
82 **Rubtsov Y**, Goryunov K, Romanov A, Suzdaltseva Y, Sharonov G, Tkachuk V. Molecular Mechanisms of Immunomodulation Properties of Mesenchymal Stromal Cells: A New Insight into the Role of ICAM-1. *Stem Cells Int* 2017; **2017**: 6516854 [PMID: 28761447 DOI: 10.1155/2017/6516854]
83 **Qiu X**, Liu J, Zheng C, Su Y, Bao L, Zhu B, Liu S, Wang L, Wang X, Wang Y, Zhao W, Zhou J, Deng Z, Jin Y. Exosomes released from educated mesenchymal stem cells accelerate cutaneous wound healing *via* promoting angiogenesis. *Cell Prolif* 2020; **53**: e12830 [PMID: 32608556 DOI: 10.1111/cpr.12830]
84 **Choudhery MS**. Strategies to improve regenerative potential of mesenchymal stem cells. *World J Stem Cells* 2021; **13**: 1845-1862 [PMID: 35069986 DOI: 10.4252/wjsc.v13.i12.1845]
85 **Negahdaripour M**, Nezafat N, Ghasemi Y. A panoramic review and in silico analysis of IL-11 structure and function. *Cytokine Growth Factor Rev* 2016; **32**: 41-61 [PMID: 27312790 DOI: 10.1016/j.cytogfr.2016.06.002]
86 **Yang W**, Zhang S, Ou T, Jiang H, Jia D, Qi Z, Zou Y, Qian J, Sun A, Ge J. Interleukin-11 regulates the fate of adipose-derived mesenchymal stem cells *via* STAT3 signalling pathways. *Cell Prolif* 2020; **53**: e12771 [PMID: 32270546 DOI: 10.1111/cpr.12771]
87 **Troncoso MF**, Ortiz-Quintero J, Garrido-Moreno V, Sanhueza-Olivares F, Guerrero-Moncayo A, Chiong M, Castro PF, García L, Gabrielli L, Corbalán R, Garrido-Olivares L, Lavandero S. VCAM-1 as a predictor biomarker in cardiovascular disease. *Biochim Biophys Acta Mol Basis Dis* 2021; **1867**: 166170 [PMID: 34000374 DOI: 10.1016/j.bbadis.2021.166170]
88 **Jarrah AA**, Schwarskopf M, Wang ER, LaRocca T, Dhume A, Zhang S, Hadri L, Hajjar RJ, Schecter AD, Tarzami ST. SDF-1 induces TNF-mediated apoptosis in cardiac myocytes. *Apoptosis* 2018; **23**: 79-91 [PMID: 29236198 DOI: 10.1007/s10495-017-1438-3]
89 **Gabrielyan A**, Quade M, Gelinsky M, Rösen-Wolff A. IL-11 and soluble VCAM-1 are important components of Hypoxia Conditioned Media and crucial for Mesenchymal Stromal Cells attraction. *Stem Cell Res* 2020; **45**: 101814 [PMID: 32334367 DOI: 10.1016/j.scr.2020.101814]
90 **Gabrielyan A**, Neumann E, Gelinsky M, Rösen-Wolff A. Metabolically conditioned media derived from bone marrow stromal cells or human skin fibroblasts act as effective chemoattractants for mesenchymal stem cells. *Stem Cell Res Ther* 2017; **8**: 212 [PMID: 28969687 DOI: 10.1186/s13287-017-0664-5]
91 **Chen Q**, Li Y, Chen Z, Du H, Wan J. Anti-VCAM 1 Antibody-Coated Mesenchymal Stromal Cells Attenuate Experimental Colitis *via* Immunomodulation. *Med Sci Monit* 2019; **25**: 4457-4468 [PMID: 31201771 DOI: 10.12659/MSM.914238]
92 **Li X**, Wang Q, Ding L, Wang YX, Zhao ZD, Mao N, Wu CT, Wang H, Zhu H, Ning SB. Intercellular adhesion molecule-1 enhances the therapeutic effects of MSCs in a dextran sulfate sodium-induced colitis models by promoting MSCs homing to murine colons and spleens. *Stem Cell Res Ther* 2019; **10**: 267 [PMID: 31443680 DOI: 10.1186/s13287-019-1384-9]
93 **Bogatcheva NV**, Coleman ME. Conditioned Medium of Mesenchymal Stromal Cells: A New Class of Therapeutics. *Biochemistry (Mosc)* 2019; **84**: 1375-1389 [PMID: 31760924 DOI: 10.1134/S0006297919110129]
94 **Pouya S**, Heidari M, Baghaei K, Asadzadeh Aghdaei H, Moradi A, Namaki S, Zali MR, Hashemi SM. Study the effects of

mesenchymal stem cell conditioned medium injection in mouse model of acute colitis. *Int Immunopharmacol* 2018; **54**: 86-94 [PMID: 29112894 DOI: 10.1016/j.intimp.2017.11.001]

95 **Kang JY**, Oh MK, Joo H, Park HS, Chae DH, Kim J, Lee HR, Oh IH, Yu KR. Xeno-Free Condition Enhances Therapeutic Functions of Human Wharton's Jelly-Derived Mesenchymal Stem Cells against Experimental Colitis by Upregulated Indoleamine 2,3-Dioxygenase Activity. *J Clin Med* 2020; **9** [PMID: 32927587 DOI: 10.3390/jcm9092913]

96 **Wu X**, Wu D, Mu Y, Zhao Y, Ma Z. Serum-Free Medium Enhances the Therapeutic Effects of Umbilical Cord Mesenchymal Stromal Cells on a Murine Model for Acute Colitis. *Front Bioeng Biotechnol* 2020; **8**: 586 [PMID: 32671030 DOI: 10.3389/fbioe.2020.00586]

97 **Jin S**, Yang C, Huang J, Liu L, Zhang Y, Li S, Zhang L, Sun Q, Yang P. Conditioned medium derived from FGF-2-modified GMSCs enhances migration and angiogenesis of human umbilical vein endothelial cells. *Stem Cell Res Ther* 2020; **11**: 68 [PMID: 32070425 DOI: 10.1186/s13287-020-1584-3]

98 **Sagaradze G**, Grigorieva O, Nimiritsky P, Basalova N, Kalinina N, Akopyan Z, Efimenko A. Conditioned Medium from Human Mesenchymal Stromal Cells: Towards the Clinical Translation. *Int J Mol Sci* 2019; **20** [PMID: 30987106 DOI: 10.3390/ijms20071656]

99 **Yang FY**, Chen R, Zhang X, Huang B, Tsang LL, Li X, Jiang X. Preconditioning Enhances the Therapeutic Effects of Mesenchymal Stem Cells on Colitis Through PGE2-Mediated T-Cell Modulation. *Cell Transplant* 2018; **27**: 1352-1367 [PMID: 30095002 DOI: 10.1177/0963689718780304]

100 **Pawitan JA**, Bui TA, Mubarok W, Antarianto RD, Nurhayati RW, Dilogo IH, Oceandy D. Enhancement of the Therapeutic Capacity of Mesenchymal Stem Cells by Genetic Modification: A Systematic Review. *Front Cell Dev Biol* 2020; **8**: 587776 [PMID: 33195245 DOI: 10.3389/fcell.2020.587776]

101 **Yang R**, Huang H, Cui S, Zhou Y, Zhang T. IFN-γ promoted exosomes from mesenchymal stem cells to attenuate colitis *via* miR-125a and miR-125b. *Cell Death Dis* 2020; **11**: 603 [PMID: 32733020 DOI: 10.1038/s41419-020-02788-0]

102 **Chen Y**, Song Y, Miao H, Xu Y, Lv M, Wang T, Hou Y. Gene delivery with IFN-γ-expression plasmids enhances the therapeutic effects of MSCs on DSS-induced mouse colitis. *Inflamm Res* 2015; **64**: 671-681 [PMID: 26153869 DOI: 10.1007/s00011-015-0845-6]

103 **Shahriar A**, Ghaleh-Aziz Shiva G, Ghader B, Farhad J, Hosein A, Parsa H. The dual role of mir-146a in metastasis and disease progression. *Biomed Pharmacother* 2020; **126**: 110099 [PMID: 32179200 DOI: 10.1016/j.biopha.2020.110099]

104 **Wu H**, Fan H, Shou Z, Xu M, Chen Q, Ai C, Dong Y, Liu Y, Nan Z, Wang Y, Yu T, Liu X. Extracellular vesicles containing miR-146a attenuate experimental colitis by targeting TRAF6 and IRAK1. *Int Immunopharmacol* 2019; **68**: 204-212 [PMID: 30654310 DOI: 10.1016/j.intimp.2018.12.043]

105 **Mao F**, Wang M, Wang J, Xu WR. The role of 15-LOX-1 in colitis and colitis-associated colorectal cancer. *Inflamm Res* 2015; **64**: 661-669 [PMID: 26194111 DOI: 10.1007/s00011-015-0852-7]

106 **Kang J**, Zhang Z, Wang J, Wang G, Yan Y, Qian H, Zhang X, Xu W, Mao F. hucMSCs Attenuate IBD through Releasing miR148b-5p to Inhibit the Expression of 15-lox-1 in Macrophages. *Mediators Inflamm* 2019; **2019**: 6953963 [PMID: 31275059 DOI: 10.1155/2019/6953963]

107 **Jiang Q**, Huang K, Lu F, Deng S, Yang Z, Hu S. Modifying strategies for SDF-1/CXCR4 interaction during mesenchymal stem cell transplantation. *Gen Thorac Cardiovasc Surg* 2022; **70**: 1-10 [PMID: 34510332 DOI: 10.1007/s11748-021-01696-0]

108 **Chen Z**, Chen Q, Du H, Xu L, Wan J. Mesenchymal stem cells and CXC chemokine receptor 4 overexpression improved the therapeutic effect on colitis *via* mucosa repair. *Exp Ther Med* 2018; **16**: 821-829 [PMID: 30112037 DOI: 10.3892/etm.2018.6233]

109 **Zheng XB**, He XW, Zhang LJ, Qin HB, Lin XT, Liu XH, Zhou C, Liu HS, Hu T, Cheng HC, He XS, Wu XR, Chen YF, Ke J, Wu XJ, Lan P. Bone marrow-derived CXCR4-overexpressing MSCs display increased homing to intestine and ameliorate colitis-associated tumorigenesis in mice. *Gastroenterol Rep (Oxf)* 2019; **7**: 127-138 [PMID: 30976426 DOI: 10.1093/gastro/goy017]

110 **Cui J**, Jia J. Natural COX-2 Inhibitors as Promising Anti-inflammatory Agents: An Update. *Curr Med Chem* 2021; **28**: 3622-3646 [PMID: 32942970 DOI: 10.2174/0929867327999200917150939]

111 **Silva DN**, Souza BSF, Azevedo CM, Vasconcelos JF, de Jesus PG, Feitoza MS, Meira CS, Carvalho GB, Cavalcante BR, Ribeiro-Dos-Santos R, Soares MBP. IGF-1-Overexpressing Mesenchymal Stem/Stromal Cells Promote Immunomodulatory and Proregenerative Effects in Chronic Experimental Chagas Disease. *Stem Cells Int* 2018; **2018**: 9108681 [PMID: 30140292 DOI: 10.1155/2018/9108681]

112 **Dave M**, Hayashi Y, Gajdos GB, Smyrk TC, Svingen PA, Kvasha SM, Lorincz A, Dong H, Faubion WA Jr, Ordog T. Stem cells for murine interstitial cells of cajal suppress cellular immunity and colitis *via* prostaglandin E2 secretion. *Gastroenterology* 2015; **148**: 978-990 [PMID: 25637652 DOI: 10.1053/j.gastro.2015.01.036]

113 **Karin N**. CXCR3 Ligands in Cancer and Autoimmunity, Chemoattraction of Effector T Cells, and Beyond. *Front Immunol* 2020; **11**: 976 [PMID: 32547545 DOI: 10.3389/fimmu.2020.00976]

114 **Nitti M**, Ivaldo C, Traverso N, Furfaro AL. Clinical Significance of Heme Oxygenase 1 in Tumor Progression. *Antioxidants (Basel)* 2021; **10** [PMID: 34067625 DOI: 10.3390/antiox10050789]

115 **Yin M**, Shen Z, Yang L, Zheng W, Song H. Protective effects of CXCR3/HO1 genemodified BMMSCs on damaged intestinal epithelial cells: Role of the p38MAPK signaling pathway. *Int J Mol Med* 2019; **43**: 2086-2102 [PMID: 30864680 DOI: 10.3892/ijmm.2019.4120]

116 **Sun D**, Cao H, Yang L, Lin L, Hou B, Zheng W, Shen Z, Song H. MiR-200b in heme oxygenase-1-modified bone marrow mesenchymal stem cell-derived exosomes alleviates inflammatory injury of intestinal epithelial cells by targeting high mobility group box 3. *Cell Death Dis* 2020; **11**: 480 [PMID: 32587254 DOI: 10.1038/s41419-020-2685-8]

117 **Watkins DJ**, Zhou Y, Matthews MA, Chen L, Besner GE. HB-EGF augments the ability of mesenchymal stem cells to attenuate intestinal injury. *J Pediatr Surg* 2014; **49**: 938-44; discussion 944 [PMID: 24888839 DOI: 10.1016/j.jpedsurg.2014.01.030]

118 **Wei J**, Zhou Y, Besner GE. Heparin-binding EGF-like growth factor and enteric neural stem cell transplantation in the prevention of experimental necrotizing enterocolitis in mice. *Pediatr Res* 2015; **78**: 29-37 [PMID: 25806717 DOI:

10.1038/pr.2015.63]
119 **Chen Y**, Zuo J, Chen W, Yang Z, Zhang Y, Hua F, Shao L, Li J, Chen Y, Yu Y, Shen Z. The enhanced effect and underlying mechanisms of mesenchymal stem cells with IL-33 overexpression on myocardial infarction. *Stem Cell Res Ther* 2019; **10**: 295 [PMID: 31547872 DOI: 10.1186/s13287-019-1392-9]
120 **Ni X**, Ou C, Guo J, Liu B, Zhang J, Wu Z, Li H, Chen M. Lentiviral vector-mediated co-overexpression of VEGF and Bcl-2 improves mesenchymal stem cell survival and enhances paracrine effects in vitro. *Int J Mol Med* 2017; **40**: 418-426 [PMID: 28627637 DOI: 10.3892/ijmm.2017.3019]
121 **Hamza AA**, Fikry EM, Abdallah W, Amin A. Mechanistic insights into the augmented effect of bone marrow mesenchymal stem cells and thiazolidinediones in streptozotocin-nicotinamide induced diabetic rats. *Sci Rep* 2018; **8**: 9827 [PMID: 29959408 DOI: 10.1038/s41598-018-28029-1]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2022 September 26; 14(9): 700-713

DOI: 10.4252/wjsc.v14.i9.700

ISSN 1948-0210 (online)

ORIGINAL ARTICLE

Basic Study

Overexpression of GATA binding protein 4 and myocyte enhancer factor 2C induces differentiation of mesenchymal stem cells into cardiac-like cells

Syeda Saima Razzaq, Irfan Khan, Nadia Naeem, Asmat Salim, Sumreen Begum, Kanwal Haneef

Specialty type: Cell biology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ahmed AA, Egypt; Exbrayat JM, France

Received: March 28, 2022
Peer-review started: March 28, 2022
First decision: June 11, 2022
Revised: June 20, 2022
Accepted: August 30, 2022
Article in press: August 30, 2022
Published online: September 26, 2022

Syeda Saima Razzaq, Kanwal Haneef, Dr. Zafar H. Zaidi Center for Proteomics, University of Karachi, Karachi 75270, Pakistan

Irfan Khan, Asmat Salim, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

Nadia Naeem, Dow Research Institute of Biotechnology & Biomedical Sciences (DRIBBS), Dow University of Health Sciences (DUHS), Ojha Campus, Karachi 75200, Pakistan

Sumreen Begum, Stem Cells Research Laboratory (SCRL), Sindh Institute of Urology and Transplantation (SIUT), Karachi 74200, Pakistan

Corresponding author: Kanwal Haneef, PhD, Assistant Professor, Dr. Zafar H. Zaidi Center for Proteomics, University of Karachi, University Road, Karachi 75270, Pakistan.
k.haneef@uok.edu.pk

Abstract

BACKGROUND

Heart diseases are the primary cause of death all over the world. Following myocardial infarction, billions of cells die, resulting in a huge loss of cardiac function. Stem cell-based therapies have appeared as a new area to support heart regeneration. The transcription factors GATA binding protein 4 (GATA-4) and myocyte enhancer factor 2C (MEF2C) are considered prominent factors in the development of the cardiovascular system.

AIM

To explore the potential of GATA-4 and MEF2C for the cardiac differentiation of human umbilical cord mesenchymal stem cells (hUC-MSCs).

METHODS

hUC-MSCs were characterized morphologically and immunologically by the presence of specific markers of MSCs *via* immunocytochemistry and flow cytometry, and by their potential to differentiate into osteocytes and adipocytes. hUC-MSCs were transfected with GATA-4, MEF2C, and their combination to direct the differentiation. Cardiac differentiation was confirmed by semiquant-

itative real-time polymerase chain reaction and immunocytochemistry.

RESULTS

hUC-MSCs expressed specific cell surface markers CD105, CD90, CD44, and vimentin but lack the expression of CD45. The transcription factors GATA-4 and MEF2C, and their combination induced differentiation in hUC-MSCs with significant expression of cardiac genes *i.e.*, *GATA-4*, *MEF2C*, *NK2 homeobox 5 (NKX2.5)*, *MHC*, and *connexin-43*, and cardiac proteins GATA-4, NKX2.5, cardiac troponin T, and connexin-43.

CONCLUSION

Transfection with GATA-4, MEF2C, and their combination effectively induces cardiac differentiation in hUC-MSCs. These genetically modified MSCs could be a promising treatment option for heart diseases in the future.

Key Words: Heart disease; GATA binding protein 4; Myocyte enhancer factor 2C; Transcription factors; Differentiation; Human umbilical cord-mesenchymal stem cells

Core Tip: Transcription factors have great potential to direct cell fate decisions during embryonic development. In this study, we investigated the overexpression of cardiac transcription factors in human umbilical cord mesenchymal stem cells to enhance their differentiation into cardiac-like cells. The synergistic effect of GATA binding protein 4 and myocyte enhancer factor 2C transcription factors increased the expression of cardiac genes and proteins. The results of this study will aid in the development of new therapeutic strategies aimed at curing heart diseases.

Citation: Razzaq SS, Khan I, Naeem N, Salim A, Begum S, Haneef K. Overexpression of GATA binding protein 4 and myocyte enhancer factor 2C induces differentiation of mesenchymal stem cells into cardiac-like cells. *World J Stem Cells* 2022; 14(9): 700-713

URL: https://www.wjgnet.com/1948-0210/full/v14/i9/700.htm

DOI: https://dx.doi.org/10.4252/wjsc.v14.i9.700

INTRODUCTION

Heart failure is the most challenging issue after myocardial infarction[1,2]. The environmental and genetic risk factors cause the deregulation of cardiomyocytes as well as endothelial, smooth muscle, and inflammatory cells of heart tissue[3]. Cardiomyocytes largely fail in adult life to divide or enter the cell cycle[4,5]. Therefore, the adult heart has a limited endogenous repair and regeneration mechanism[6-8]. Current interventions rely on heart transplantation, mechanical assistance devices, and medicinal therapies for the management of damaged organ. However, these options cannot revert the normal functioning of the heart. The future therapeutic strategy for cardiac diseases is to regenerate damaged tissue for restoring complete heart function[9,10].

Cell based therapies are promising for damaged heart tissue. Stem cells possess the remarkable potential to stimulate endogenous myocardial repair and regeneration processes[11-15]. However, the low viability of transplanted stem cells due to inadequate supply of blood and inflamed myocardium has been a major challenge[12-14]. Adult mesenchymal stem cells (MSCs) have the potential to make bone, muscle, nerve, cardiac, and fat cells[16,17]. Furthermore, MSCs help in the formation of new blood vessels, induce apoptotic resistance, and provide anti-fibrotic effects[18,19]. One of the recently employed innovative approaches is the use of forward programming with tissue type-specific transcription factors for the differentiation of stem cells[20].

The successful cell fate reprogramming requires a temporospatial expression pattern of transcription factors[21]. Heart development is a complex process that requires the coordination of a series of events such as specification, proliferation, and differentiation[22]. Cardiac transcription factors, including GATA binding protein 4 (GATA-4), myocyte enhancer factor 2A (MEF2A), NK2 homeobox 5 (NKX2.5), and serum response factor (Srf), have a paradoxical role in the differentiation and homeostasis of myocardial cells[23]. It has been documented that three cardiac transcription factors, GATA-4, NKX2.5, and T-Box transcription factor 5 (TBX5), programmed extra-cardiac mesoderm of mouse embryo into cardiac tissue[24]. Also, a combination of GATA-4, NKX2.5, TBX5, and BAF60C can induce the differentiation of embryonic stem cells into cardiac lineage[25]. Altogether, these research studies display that transcription factor mediated stem cell reprogramming is a valuable strategy that directs cardio-

myogenic differentiation of various stem cell types.

The current study aimed to examine the effects of overexpressing two cardiac transcription factors, GATA-4 and MEF2C, in cardiac differentiation of human umbilical cord MSCs (hUC-MSCs). After introducing the transcription factors either individually or in combination, hUC-MSCs were analyzed for the expression of cardiac genes and proteins. These genetically modified MSCs could be a promising treatment option for cardiovascular diseases.

MATERIALS AND METHODS

Ethics committee approval

The current research project was approved by the institutional bioethical committee of University of Karachi (protocol #: ICB KU-92/2020).

Human umbilical cord collection

Human umbilical cords (n = 12) were collected from healthy pregnant females at the Dow University of Health Science, OJHA campus, Karachi, Pakistan after obtaining the consent from the donors.

Isolation and propagation of hUC-MSCs

Human umbilical cord tissue was longitudinally cut and thoroughly washed with sterile phosphate-buffered saline (PBS). The human cord tissue was cut into 2-5 mm in size and placed in 1 × (0.25%) trypsin (GIBCO, United States) for 20 min at 37 °C. Partially digested cord tissues were kept in a T-25 tissue culture flask having 3-5 mL of DMEM (GIBCO, United States) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin/streptomycin, and 1 mmol sodium pyruvate. Explants were placed at 37 °C with 5% CO_2 (Heracell, United States). The medium was changed every third day. MSCs attached to the tissue culture flask during 15-20 d of the first culture. After adhesion of the MSCs, tissues were discarded and fresh DMEM was added for the proliferation of cells. Once the MSCs reached 70% to 80% confluence, they were detached using 1 × (0.25%) trypsin. hUC-MSCs at passage (P) 1-2 were used for experiments.

Immunocytochemistry

Isolated hUC-MSCs were characterized by immunocytochemistry to detect the specific markers of MSCs. Briefly, 4% paraformaldehyde (PFA) was added to the cells and then incubated with 0.1% Triton X-100. The permeabilized cells were then kept in a blocking solution for 1 h. After incubation, the solution was discarded and cells were kept at 4 °C with anti-mouse primary antibodies against CD90, CD105, vimentin, CD44, and CD45. After overnight incubation, cells were thoroughly washed 4-5 times with PBS. Alexa fluor 488 conjugated goat anti-mouse secondary antibody was added to each well. The negative control cells were incubated only with the secondary antibody. DAPI (4',6-diamidino-2-phenylindole) was used to stain the cell nuclei. Lastly, cells were mounted and observed under a fluorescence microscope (NIE, Nikon, Japan).

Flow cytometry

MSCs were washed 2-3 times with PBS and incubated with dissociation buffer at 37 °C for 40 min. The cells were pelleted down through centrifugation and then the cell pellet was mixed in FACS solution containing 1% BSA, 1 mmol EDTA, and 0.1% Na-azide. The tubes were centrifuged for 5 min and then the blocking solution was added to all the tubes. Primary antibodies against CD44, CD90, and CD73 were added and the tubes were incubated at 4 °C. After washing with FACS solution, Alexa fluor 488 conjugated goat anti-mouse secondary antibody was added. Unlabeled and isotype labeled cells were used as controls. Data were analyzed using BD FACS Diva software.

Adipogenic and osteogenic differentiation

Approximately 4 × 10^5 hUC-MSCs were seeded in a 6-well plate for 24 h. After confirming cell proliferation, cells were washed with sterile PBS. For osteogenesis, low glucose DMEM supplemented with 10 mmol glycerol-2-phosphate, 0.2 mmol ascorbic acid, 0.1 μmol dexamethasone, 10% FBS, 100 μg/mL streptomycin, 100 units/mL penicillin, and 2 mmol L-glutamine were added into the cell culture plate. The medium was replaced every 4th day till 21 d. After the completion of 21 d incubation period, ice cold 75% ethanol was used for cell fixation, and then the cells were stained with 2% Alizarin stain.

For adipogenesis, hUC-MSCs were cultured in adipogenic induction and maintenance medium for 21 d. Adipogenic induction medium contains 10 μg/mL insulin, 100 μmol indomethacin, 1 μmol dexamethasone, 10% FBS, 100 μg/mL streptomycin, and 100 units/mL penicillin in low glucose DMEM. After 21 d, 4% PFA was used for cell fixation and then cells were stained with 0.5% Oil Red O. Finally, images were taken under a phase contrast microscope (CKX41, Olympus, Japan).

hUC-MSC transfection

GATA-4 and MEF2C plasmids were purchased from Addgene (plasmid No. 46030 and No. 46031, respectively). Plasmid DNA was isolated by using a maxiprep plasmid DNA isolation kit (Thermo Scientific, United States). Briefly, *Escherichia coli* were harvested by centrifugation at 5000 × g. The pellet was mixed in resuspension solution and then lysis solution was added. The suspension was incubated at room temperature for 3 min and a neutralization solution followed by endotoxin binding reagent was added to the tube. The tube was incubated at room temperature for a further 5 min and 96% ethanol was added. The supernatant was collected through centrifugation, mixed with 96% ethanol, and then shifted to the purification column. The tube was centrifuged at 2000 × g for 3 min. Wash solution 1 was added to the column and centrifuged at 3000 × g. This step was repeated with wash solution 2. The plasmid DNA was eluted in elution buffer and quantified using a nano-drop spectrophotometer. hUC-MSCs were transfected separately with GATA-4 and MEF2C, and co-transfected with 1 μg each of GATA-4 and MEF2C plasmids using lipofectamine 3000 kit (Invitrogen, United States). Briefly, the plasmid vector (1 μg for GATA-4 or MEF2C) was diluted in serum free DMEM, and 2 μL of P3000 reagent was added per 1 μg of plasmid DNA. Lipofectamine TM 3000 reagent and DNA were mixed and kept at room temperature for 15 min. Cells at 70%-80% confluence were incubated with DNA-lipid complex at 37 °C for 24 h. After 24 h, lipofectamine was replaced with FBS containing DMEM. The cells were kept for 2 wk at 37 °C using an air jacketed CO_2 incubator. The medium was changed every 3 to 4 d. The following experimental groups were used in this study: Untreated control, GATA-4 transfected, MEF2C transfected, and combination group of GATA-4 + MEF2C transfected hUC-MSCs.

Gene expression analysis of transfected hUC-MSCs

The overexpression of the *GATA-4* and *MEF2C* genes in transfected hUC-MSCs was confirmed by semiquantitative real-time polymerase chain reaction (RT-PCR). RNA was extracted from transfected and control hUC-MSCs using TRIzol reagent. For RNA isolation, cells were harvested and the pellet was gently mixed with TRIzol reagent. In the next step, chloroform was added to the tube and incubated at room temperature for 15 min. The cell suspension was centrifuged at 12000 × g for 15 min. Isopropyl alcohol was added to the separated aqueous phase followed by centrifugation at 12000 × g. The RNA pellet was air dried and then resuspended in RNAase-free water. The RNA absorbance was calculated at 260 nm. cDNA was synthesized using a cDNA synthesis kit (Invitrogen, United States) and then amplified using primers corresponding to *GATA-4* and *MEF2C* genes. Human *beta-actin* was used as a housekeeping gene. Reverse transcription reaction products were initially denatured for 30 s at 94 °C, followed by 40 cycles of amplification: Denaturation at 94 °C for 3 s and annealing at 60 °C for 30s. Primer sequences and melting temperatures of each gene are enlisted in Table 1.

Analysis of cardiac genes and proteins

For gene expression, RT-PCR of untreated and transfected hUC-MSCs was performed at day 14 of transfection. For cardiac protein expression, immunocytochemistry staining of untreated and transfected hUC-MSCs was performed also on day 14 of transfection. Primary antibodies for cardiac specific proteins, *i.e.*, GATA-4, connexin-43, NKX2.5, and cTnT, were used. The negative control cells were incubated only with the secondary antibody. Finally, cells were mounted and images were taken under a fluorescence microscope (NIE, Nikon, Japan). The fluorescence intensities were calculated through Image J software (NIH, United States).

Statistical analysis

Data were analyzed by using IBM SPSS Statistics 20 software. One way ANOVA and Tukey's post hoc test were used for comparisons among multiple groups. All data were collected from three independent experiments. A P value less than 0.05 ($^aP < 0.05$) was considered statistically significant.

RESULTS

Morphological features of MSCs derived from human umbilical cord tissue

Adherent cells started to grow during 15 d to 20 d of isolation and are termed P0 cells, as shown in Figure 1. The P0 cells were sub-cultured once they reached 80% confluence and termed P1 cells. The hUC-MSCs appeared in colonies and showed a fibroblast-like morphology (Figure 1). P1 to P2 cells were used in this study.

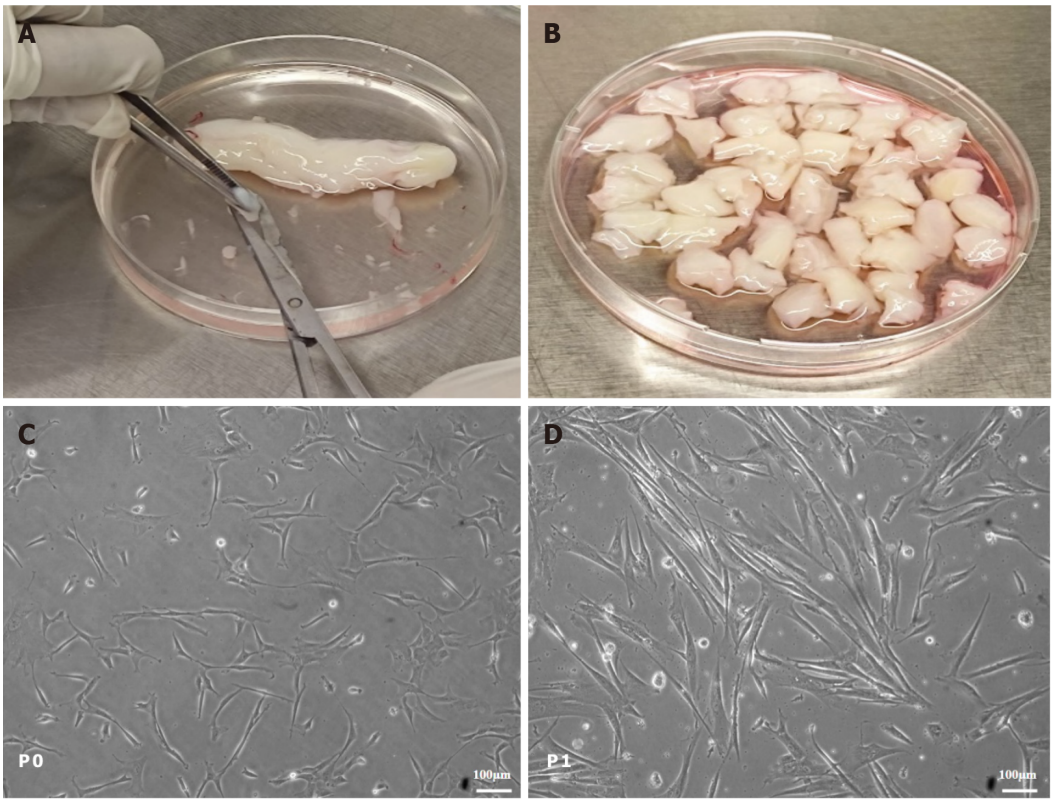
Characterization of hUC-MSCs

Immunocytochemistry analysis showed positive expression of the MSC markers CD105, CD90, CD44, and vimentin, while CD45, a hematopoietic marker, was not expressed in these cells (Figure 2A). The immunophenotypic analysis showed positive expression of CD90, CD73, and CD44 in hUC-MSCs (Figure 2B). The osteogenic and adipogenic differentiation was confirmed, respectively, by Alizarin Red staining which revealed mineral deposits, and Oil Red O staining which revealed lipid droplets

Table 1 Primer sequence and annealing temperature of cardiac genes

Gene	Primer sequence (5'-3')	Annealing temperature (°C)
Beta-actin	Forward: 5′-TGGGCATGGGTCAGAAGGATTC-3′	60
	Reverse: 5′-AGGTGTGGTGCCAGATTTTCTC-3′	
Myocyte enhancer factor 2C	Forward: 5′-CGAGATGCCAGTCTCCATCC-3′	60
	Reverse: 5′-CAGAGAAGGGTGAGCCAGTG-3′	
NKX2.5	Forward: 5′-AGTGTGCGTCTGCCTTTCC-3′	60
	Reverse: 5′-CACAGCTCTTTCTTTTCGGCTC-3′	
MHC	Forward: 5′-GACAGGTGCAGCAAAA CAGG-3′ Reverse: 5′-AAGGGTATCCTGCAACTGCC-3′	60
Connexin-43	Forward: 5′-CTTCATGCTGGTGGTGTCC-3′	60
	Reverse: 5′-ACCACTGGTCGCATGGTAAG-3′	
GATA-4	Forward: 5′-CTGCCCTCCGTCTTCTGC-3′	60
	Reverse: 5′-CTCGCAGGTCAAGGAGCC-3′	

NKX2.5: NK2 homeobox 5; MEF2C: Myocyte enhancer factor 2C; MHC: Myosin heavy chain; GATA-4: GATA binding protein 4.



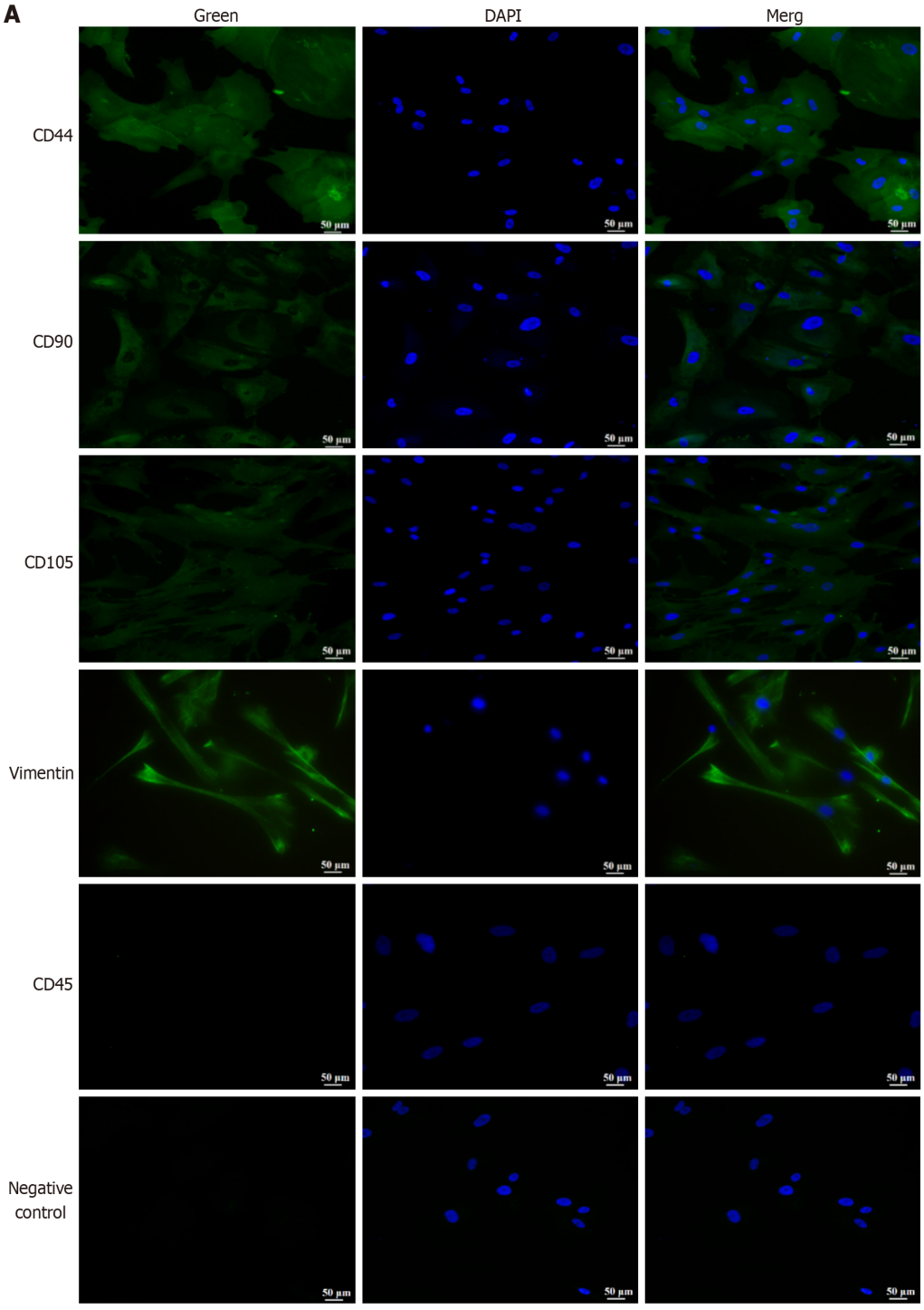
DOI: 10.4252/wjsc.v14.i9.700 **Copyright** ©The Author(s) 2022.

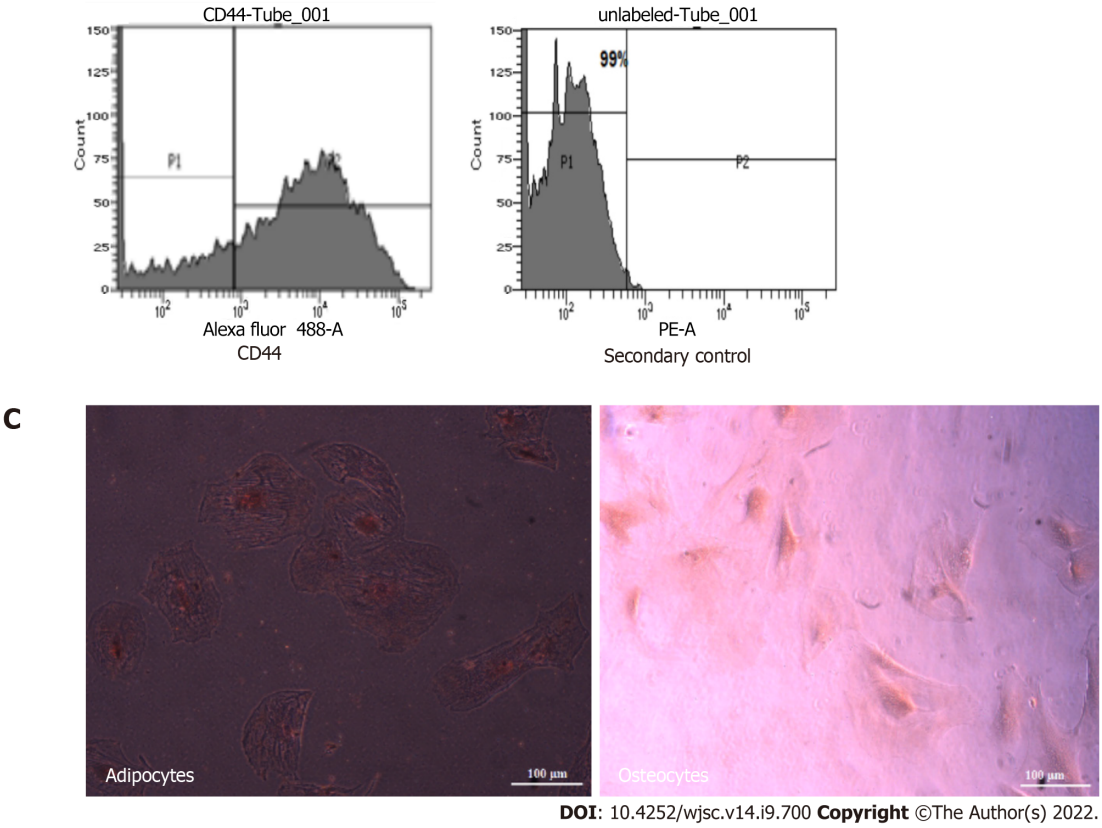
Figure 1 Isolation and morphology of human umbilical cord mesenchymal stem cells. A-D: A stepwise method of isolation and proliferation of human umbilical cord mesenchymal stem cells (hUC-MSCs), which show a spindle-shaped fibroblast-like cell morphology under the phase contrast microscope at P0 and P1. All images were captured under a phase contrast microscope (scale bar: 100 μm).

(Figure 2C).

Molecular analysis of transfected hUC-MSCs

hUC-MSCs were successfully transfected with *GATA-4* and *MEF2C* genes. RT-PCR analysis showed a significant increase in *GATA-4* and *MEF2C* expression after 24 h of transfection compared with the control (Figure 3).


A
Green
DAPI
Merg
CD44
CD90
CD105
Vimentin
CD45
Negative control
50 μm
B
CD90-Tube_001
CD73-Tube_001
Count
P1
P2
Alexa fluor 488-A
CD90
PE-A
CD73



DOI: 10.4252/wjsc.v14.i9.700 **Copyright** ©The Author(s) 2022.

Figure 2 Human umbilical cord mesenchymal stem cell characterization by immunocytochemical analysis, flow cytometry, and lineage differentiation assays. A: Immunocytochemsitry of human umbilical cord mesenchymal stem cell (hUC-MSC) showing positive expression of CD44, CD90, CD105, and vimentin, and negative expression of CD45, a hematopoietic marker. Images were captured under a fluorescence microscope (scale bar: 50 µm); B: Flow cytometry of hUC-MSCs showing positive expression of CD44, CD73, and CD90. Data were analyzed using BD FACS Diva software; C: Adipogenic and osteogenic lineage differentiation of hUC-MSCs. Images were captured under a phase contrast microscope (scale bar: 100 µm).

Morphological changes and gene expression analysis of transfected hUC-MSCs

After 14 d of culture, the transfected cells displayed extended cytoplasmic processes and myotube like structures which are the typical features of cardiomyocytes (Figure 4A). hUC-MSCs transfected with GATA-4, MEF2C, and their combination showed significant expression of cardiac genes including *MEF2C*, *NKX2.5*, *GATA-4*, *connexin-43*, and *myosin heavy chain (MHC)* (Figure 4B). Moreover, the combination group for the evaluation of the synergistic effect of both transcription factors showed significant expression of cardiac genes as compared to the individual groups (Figure 4B).

Expression of cardiac proteins in transfected hUC-MSCs

Cardiac differentiation of transfected hUC-MSCs was further confirmed using immunocytochemistry. hUC-MSCs transfected with GATA-4, MEF2C, or their combination exhibited positive expression of cardiac specific proteins, including connexin-43, cTnT, GATA-4, and NKX2.5 as compared to the untreated control at day 14 (Figure 5A). Moreover, the fluorescence intensity of hUC-MSCs transfected with GATA-4, MEF2C, and their combination was also calculated using Image J software. Statistical analysis showed significant up-regulation of GATA-4, connexin-43, and NKX2.5 in all three treatment groups as compared to the untreated control. However, the late cardiac marker cTnT was not up-regulated at day 14 (Figure 5B).

DISCUSSION

This study determined the effects of two cardiac transcription factors, GATA-4 and MEF2C, on the differentiation of hUC-MSCs towards cardiac lineage *in vitro*. GATA-4 is an important transcription factor that regulates the proliferation, survival, and fate commitment of many cell types[26]. Moreover, GATA-4 plays a vital role in the process of heart development[27]. The myocyte enhancer factor 2C (MEF2C) acts as a transcriptional regulator in cardiovascular growth[28]. It is demonstrated by various studies that MEF2C acts together with GATA factors to induce gene transcription in cardiomyocytes [27]. Based on their widely documented role in the structure and function of the heart, we hypothesized

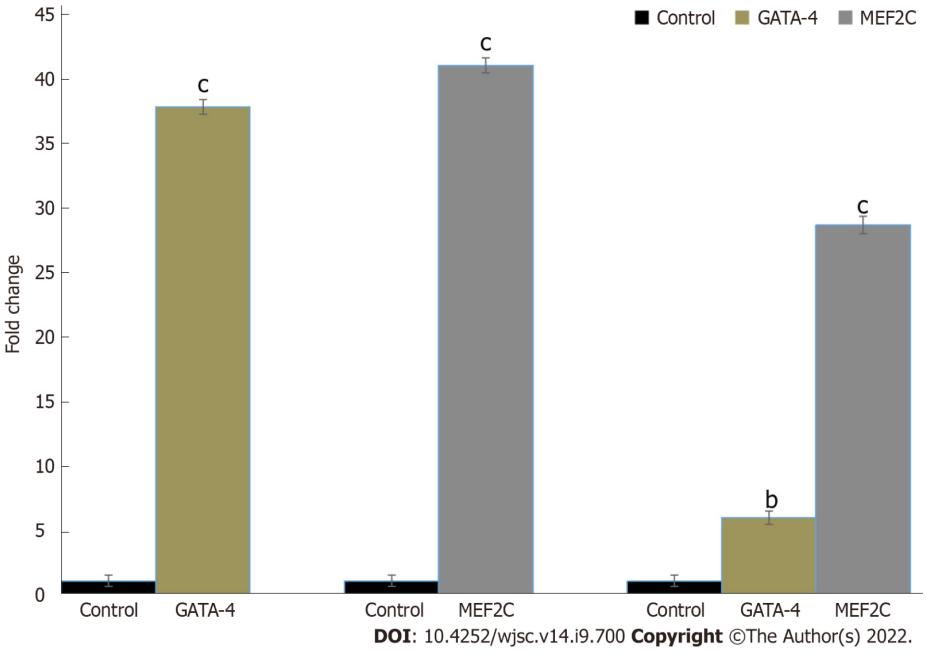


Figure 3 Gene expression analysis of GATA binding protein 4 and myocyte enhancer factor 2C transfected human umbilical cord mesenchymal stem cells. Semiquantitative real-time polymerase chain reaction (RT-PCR) analysis was performed to show the gene expression levels of GATA binding protein 4 and myocyte enhancer factor 2C transfected mesenchymal stem cells, separately and in combination, in comparison to the control. Results are expressed as the mean ± SE (n = 3). Differences between groups are considered statistically significant where $^{b}P < 0.01$ and $^{c}P < 0.001$. GATA-4: GATA binding protein 4; MEF2C: Myocyte enhancer factor 2C.

that GATA-4 and MEF2C overexpression may have the potential to induce the differentiation of hUC-MSCs into cardiac-like cells.

In this study, hUC-MSCs were isolated by the explant method[29]. The characterization studies of isolated cells were performed according to the standard criteria of the International Society for Stem Cell Research (ISSCR)[30]. The isolated cells showed a fibroblast-like morphology and positive expression of CD105, CD90, CD44, and vimentin, whereas they lack the expression of the hematopoietic marker CD45. MSCs specific markers CD73, CD90, and CD44 were also verified by flow cytometry analysis. Moreover, cord derived MSCs showed the differentiation potential of adipocytes and osteocytes. The results of our study confirmed that the cord derived cells possess the main characteristics of MSCs. Next, we analyzed the overexpression of *GATA-4* and *MEF2C* mRNA in control and transfected hUC-MSCs. The expression of *GATA-4* and *MEF2C* was maximum 24 h after transfection. Based on these gene expression data, we selected 24 h transfected hUC-MSCs for further experiments. hUC-MSCs were transfected with GATA-4 and MEF2C separately and in combination for 24 h, and then their cardiac differentiation potential at day 14 was analyzed. We observed elongated cells with extended cytoplasmic processes in the transfected groups in comparison with the control group. The transfected cells had a morphology similar to cardiomyocytes and these results are also in line with earlier studies[31,32].

The cardiac differentiation of transfected cells at day 14 was analyzed *via* analysis of mRNA expression of early and late cardiac specific markers, such as *GATA-4, MEF2C, NKX2.5, connexin-43,* and *MHC*. Cardiac markers were initiated to express in the GATA-4 and MEF2C transfected cells, while their significant up-regulation was prominent in the combination group. The cardiac transcription factor GATA-4 facilitates the binding of various transcriptional factors and co-activators including GATA-6, NKX2.5, Srf, MEF2, dHAND, YY1, and NFAT[33]. MEF2C participates in the growth and maturation of myocardial cells with GATA-4[34]. It has been found that the overexpression of transcription factors induces cardiomyocyte differentiation in stem cells[35,36]. The combination of precardiac mesodermal transcription factors (Csx/NKX2.5 and GATA-4) has been reported to induce cardiac differentiation of 9-15c stem cells[37]. It has been found that GATA-4, MEF2C, and TBX5 generated cardiomyocyte like cells from mouse heart fibroblast[38]. The gene expression data revealed that GATA-4, MEF2C, and their combination were capable of directing stem cell fate into cardiomyocytes *in vitro*. Additionally, in the combination group, the significantly higher expression of cardiac specific genes indicates their synergistic effect on cardiac differentiation.

To complement gene expression data, we analyzed cardiac specific proteins in the GATA-4, MEF2C, their combination, and control groups. The combination group showed significant up-regulation of connexin-43, NKX2.5, and GATA-4 proteins at day 14 of transfection. The transcription factor NKX2.5 is expressed at the early and late stages of heart development[39]. NKX2.5 transcription is regulated by binding with GATA-4 and MEF2C[40,41]. The late stage marker troponin T regulates cardiac rhythm and maintains thin filaments in cardiac and skeletal muscles[42]. The heart rhythm regulation and

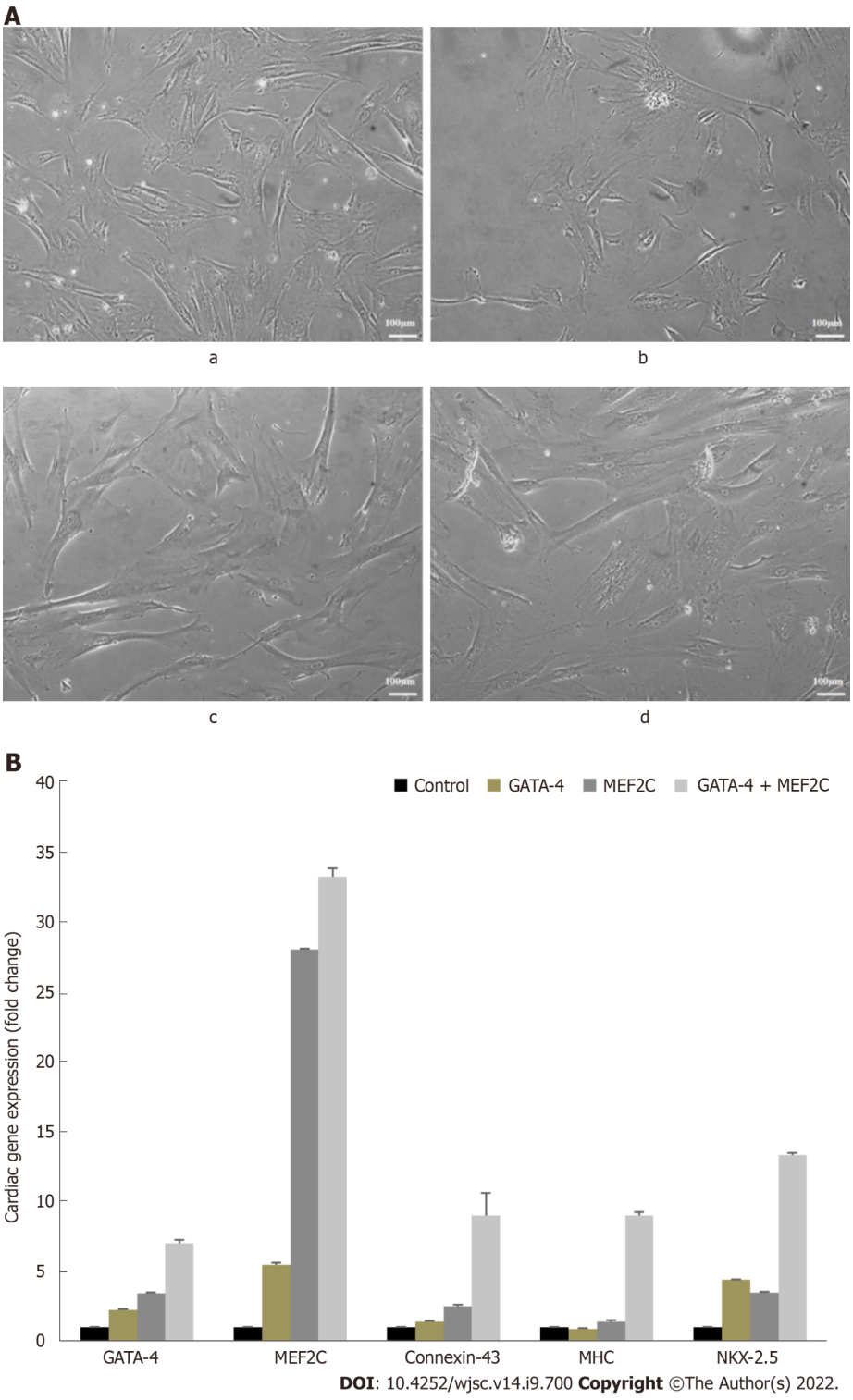
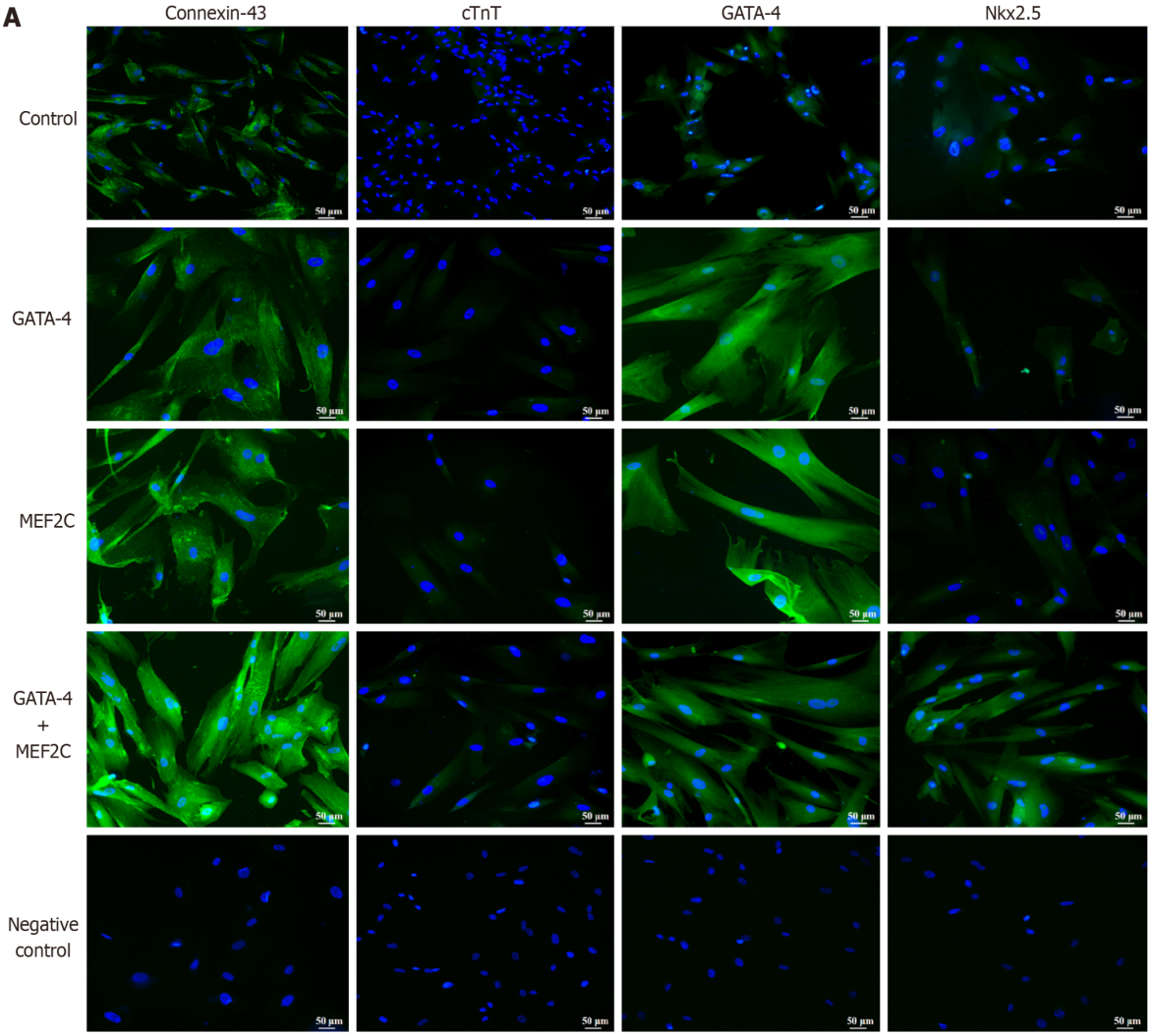


Figure 4 Morphological changes and cardiac-specific gene expression in transfected human umbilical cord mesenchymal stem cells. A: Images showing human umbilical cord mesenchymal stem cells trasnfected with (b) GATA binding protein 4 (GATA-4), (c) myocyte enhancer factor 2C (MEF2C), and (d) GATA-4 + MEF2C, and (a) the corresponding untreated control. All images were captured at day 14 under a phase contrast microscope (scale bar: 100 μm); B: Bar diagrams showing fold change analysis of cardiac gene expression by semiquantitative real-time polymerase chain reaction (RT-PCR) in the transfected cells in comparison to the control cells after 14 d of culture. Results are expressed as the mean ± SE (n = 3). Differences between groups are considered statistically significant where $^{a}P < 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$. GATA-4: GATA binding protein 4; MEF2C: Myocyte enhancer factor 2C; MHC: Myosin heavy chain; NKX2.5: NK2 homeobox 5.

coordinated contraction are controlled by a complex network of interconnected cardiomyocytes. Gap junction proteins help cardiomyocytes to communicate with their surrounding cells[43]. Connexin-43 is the major connexin protein involved in the propagation of electrical signals essential for the structural and functional maintenance of cardiac cells[44].



DOI: 10.4252/wjsc.v14.i9.700

Figure 5 Cardiac-specific protein expression in transfected human umbilical cord mesenchymal stem cells. A: Fluorescence images showing

human umbilical cord mesenchymal stem cells (hUC-MSCs) transfected with GATA binding protein 4 (GATA-4) and myocyte enhancer factor 2C (MEF2C), separately and in combination, in comparison to the control cells (scale bar: 50 μm); B: Bar diagrams showing quantification of positive cells using ImageJ software. Also shown is the comparison between the individual and combination groups. Results are expressed as the mean ± SE (n = 5). Differences between groups are considered statistically significant where $^{a}P < 0.05$ and $^{c}P < 0.001$. GATA-4: GATA binding protein 4; MEF2C: Myocyte enhancer factor 2C; CTnT: Cardiac troponin T; NKX2.5: NK2 homeobox 5; NS: Not significant.

Collectively, the results of the current study demonstrate that hUC-MSCs overexpressing GATA-4 and/or MEF2C have the potential to generate cardiac-like cells. These genetically modified MSCs may be used as a new therapeutic approach for the regeneration of heart tissue.

CONCLUSION

It is concluded from this study that overexpression of the cardiac transcription factors in hUC-MSCs enhanced their differentiation potential into cardiac-like cells. The expression of early and late cardiac genes was significantly higher in all treatment groups. However, the combination group showed enhanced synergistic effect on cardiac differentiation. GATA-4 and MEF2C delivery seems to have the potential for the development of a cell-based treatment approach for cardiovascular diseases. However, further research is needed to explore the therapeutic effects of transfected hUC-MSCs in *in vivo* models.

ARTICLE HIGHLIGHTS

Research background

Myocardial infarction is the leading cause of death worldwide. Following myocardial infarction, billions of cardiomyocytes die, resulting in a significant loss in cardiac function. Cell-based therapies have emerged as a new area to support heart regeneration. GATA binding protein 4 (GATA-4) and myocyte enhancer factor 2C (MEF2C) are considered important transcription factors in the formation of cardiac cells during the embryonic development.

Research motivation

Stem cell based therapies are considered a promising approach for repairing the damaged heart. However, the underlying mechanisms that control stem cell mediated cardiac cell fate decisions are still poorly understood. Since GATA-4 and MEF2C are the critical regulators of cardiac differentiation, use of these factors for transfection of mesenchymal stem cells (MSCs) may enhance the potential of these stem cells for cardiac differentiation.

Research objectives

Considering the critical role of cardiac transcription factors in maintaining the structure and function of the heart during the development process, their role in cardiac differentiation is highly anticipated. These genetically modified MSCs could be a promising future therapeutic option for heart diseases.

Research methods

Human umbilical cord-MSCs (hUC-MSCs) were isolated and characterized morphologically and immunologically. The cord derived MSCs were identified by the presence of specific markers *via* immunocytochemistry and flow cytometry, and by their potential for osteogenic and adipogenic differentiation. hUC-MSCs were transfected with GATA-4, MEF2C, and their combination to direct cardiac differentiation. Cardiac differentiation was confirmed by semiquantitative real-time polymerase chain reaction and immunocytochemistry.

Research results

GATA-4, MEF2C, and their combination induced the differentiation of hUC-MSCs with significant expression of cardiac genes and proteins. Moreover, myotube like structure, which is the main characteristic of cardiomyocytes, was also observed in the transfected cells.

Research conclusions

Overexpression of GATA-4 and MEF2C in hUC-MSCs induces the differentiation of stem cells into cardiac-like cells. This study is an attempt to provide deeper insights into the mechanism of transcription factors in the cardiac differentiation of stem cells.

Research perspectives

The knowledge of the current study offers a promising therapeutic approach to improve treatment strategies for heart diseases. The genetically modified MSCs may serve as an ideal source for cardiac tissue repair and regeneration.

FOOTNOTES

Author contributions: Razzaq SS performed the experiments, did data analysis, and wrote the first draft of the manuscript; Khan I co-supervised the research study and assisted in transfection studies; Naeem N assisted in qPCR; Begum S assisted in immunocytochemistry; Salim A assisted in all *in vitro* studies; Haneef K conceived the idea, supervised the research study, and finalized the manuscript.

Supported by the Higher Education Commission (HEC), Pakistan Scholarship for Ph.D. Studies to Razzaq SS, No. 520-148390-2BS6-011.

Institutional review board statement: The study was reviewed and approved by the institutional bioethical committee of University of Karachi (No. ICB KU-92/2020).

Conflict-of-interest statement: The authors confirm that this article has no conflict of interest to report.

Data sharing statement: No additional data are available.

Country/Territory of origin: Pakistan

ORCID number: Syeda Saima Razzaq 0000-0003-1867-9034; Irfan Khan 0000-0003-1878-7836; Nadia Naeem 0000-0003-1970-7340; Asmat Salim 0000-0001-5181-0458; Sumreen Begum 0000-0002-2236-1726; Kanwal Haneef 0000-0002-4725-4281.

S-Editor: Chen YL
L-Editor: Wang TQ
P-Editor: Chen YX

REFERENCES

1 **Castellan RFP**, Meloni M. Mechanisms and Therapeutic Targets of Cardiac Regeneration: Closing the Age Gap. *Front Cardiovasc Med* 2018; **5**: 7 [PMID: 29459901 DOI: 10.3389/fcvm.2018.00007]
2 **Hodgkinson CP**, Kang MH, Dal-Pra S, Mirotsou M, Dzau VJ. MicroRNAs and Cardiac Regeneration. *Circ Res* 2015; **116**: 1700-1711 [PMID: 25953925 DOI: 10.1161/CIRCRESAHA.116.304377]
3 **Bauer AJ**, Martin KA. Coordinating Regulation of Gene Expression in Cardiovascular Disease: Interactions between Chromatin Modifiers and Transcription Factors. *Front Cardiovasc Med* 2017; **4**: 19 [PMID: 28428957 DOI: 10.3389/fcvm.2017.00019]
4 **Zacchigna S**, Giacca M. Extra- and intracellular factors regulating cardiomyocyte proliferation in postnatal life. *Cardiovasc Res* 2014; **102**: 312-320 [PMID: 24623280 DOI: 10.1093/cvr/cvu057]
5 **Zhang D**, Wang Y, Lu P, Wang P, Yuan X, Yan J, Cai C, Chang CP, Zheng D, Wu B, Zhou B. Author Correction: REST regulates the cell cycle for cardiac development and regeneration. *Nat Commun* 2018; **9**: 167 [PMID: 29330540 DOI: 10.1038/s41467-017-02617-7]
6 **Karakikes I**, Senyei GD, Hansen J, Kong CW, Azeloglu EU, Stillitano F, Lieu DK, Wang J, Ren L, Hulot JS, Iyengar R, Li RA, Hajjar RJ. Small molecule-mediated directed differentiation of human embryonic stem cells toward ventricular cardiomyocytes. *Stem Cells Transl Med* 2014; **3**: 18-31 [PMID: 24324277 DOI: 10.5966/sctm.2013-0110]
7 **Broughton KM**, Wang BJ, Firouzi F, Khalafalla F, Dimmeler S, Fernandez-Aviles F, Sussman MA. Mechanisms of Cardiac Repair and Regeneration. *Circ Res* 2018; **122**: 1151-1163 [PMID: 29650632 DOI: 10.1161/CIRCRESAHA.117.312586]
8 **Haneef K**, Ali A, Khan I, Naeem N, Jamall S, Salim A. Role of interleukin-7 in fusion of rat bone marrow mesenchymal stem cells with cardiomyocytes in vitro and improvement of cardiac function in vivo. *Cardiovasc Ther* 2018; **36**: e12479 [PMID: 30451388 DOI: 10.1111/1755-5922.12479]
9 **Foglia MJ**, Poss KD. Building and re-building the heart by cardiomyocyte proliferation. *Development* 2016; **143**: 729-740 [PMID: 26932668 DOI: 10.1242/dev.132910]
10 **Lazaros G**, Oikonomou E, Tousoulis D. Established and novel treatment options in acute myocarditis, with or without heart failure. *Expert Rev Cardiovasc Ther* 2017; **15**: 25-34 [PMID: 27858465 DOI: 10.1080/14779072.2017.1262764]

11 **Saheli M**, Pirhajati Mahabadi V, Mesbah-Namin SA, Seifalian A, Bagheri-Hosseinabadi Z. DNA methyltransferase inhibitor 5-azacytidine in high dose promotes ultrastructural maturation of cardiomyocyte. *Stem Cell Investig* 2020; **7**: 22 [PMID: 33437842 DOI: 10.21037/sci-2020-007]

12 **Gnecchi M**. Cell Therapy for Heart Regeneration: Learning from the Past to Build a Brighter Future. *Stem Cells Transl Med* 2018; **7**: 702-704 [PMID: 30194808 DOI: 10.1002/sctm.18-0126]

13 **Abdelwahid E**, Kalvelyte A, Stulpinas A, de Carvalho KA, Guarita-Souza LC, Foldes G. Stem cell death and survival in heart regeneration and repair. *Apoptosis* 2016; **21**: 252-268 [PMID: 26687129 DOI: 10.1007/s10495-015-1203-4]

14 **Hu M**, Guo G, Huang Q, Cheng C, Xu R, Li A, Liu N, Liu S. The harsh microenvironment in infarcted heart accelerates transplanted bone marrow mesenchymal stem cells injury: the role of injured cardiomyocytes-derived exosomes. *Cell Death Dis* 2018; **9**: 357 [PMID: 29500342 DOI: 10.1038/s41419-018-0392-5]

15 **Dixit P**, Katare R. Challenges in identifying the best source of stem cells for cardiac regeneration therapy. *Stem Cell Res Ther* 2015; **6**: 26 [PMID: 25886612 DOI: 10.1186/s13287-015-0010-8]

16 **Shen H**, Wang Y, Zhang Z, Yang J, Hu S, Shen Z. Mesenchymal Stem Cells for Cardiac Regenerative Therapy: Optimization of Cell Differentiation Strategy. *Stem Cells Int* 2015; **2015**: 524756 [PMID: 26339251 DOI: 10.1155/2015/524756]

17 **Galli D**, Vitale M, Vaccarezza M. Bone marrow-derived mesenchymal cell differentiation toward myogenic lineages: facts and perspectives. *Biomed Res Int* 2014; **2014**: 762695 [PMID: 25054145 DOI: 10.1155/2014/762695]

18 **Mao Q**, Lin C, Gao J, Liang X, Gao W, Shen L, Kang L, Xu B. Mesenchymal stem cells overexpressing integrin-linked kinase attenuate left ventricular remodeling and improve cardiac function after myocardial infarction. *Mol Cell Biochem* 2014; **397**: 203-214 [PMID: 25134935 DOI: 10.1007/s11010-014-2188-y]

19 **Williams AR**, Hatzistergos KE, Addicott B, McCall F, Carvalho D, Suncion V, Morales AR, Da Silva J, Sussman MA, Heldman AW, Hare JM. Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. *Circulation* 2013; **127**: 213-223 [PMID: 23224061 DOI: 10.1161/CIRCULATIONAHA.112.131110]

20 **Al-Maqtari T**, Hong KU, Vajravelu BN, Moktar A, Cao P, Moore JB 4th, Bolli R. Transcription factor-induced activation of cardiac gene expression in human c-kit+ cardiac progenitor cells. *PLoS One* 2017; **12**: e0174242 [PMID: 28355297 DOI: 10.1371/journal.pone.0174242]

21 **Laemmle LL**, Cohen JB, Glorioso JC. Constitutive Expression of GATA4 Dramatically Increases the Cardiogenic Potential of D3 Mouse Embryonic Stem Cells. *Open Biotechnol J* 2016; **10**: 248-257 [PMID: 27441042 DOI: 10.2174/1874070701610010248]

22 **Grunert M**, Dorn C and Rickert-Sperling S. Congenital Heart Diseases: The Broken Heart. In: Rickert-Sperling, S., Kelly, R., Driscoll, D. (eds), Cardiac transcription factors and regulatory networks. ViennaL Springer, 2016: 139-152 [DOI: 10.1007/978-3-7091-1883-2_12]

23 **Schlesinger J**, Schueler M, Grunert M, Fischer JJ, Zhang Q, Krueger T, Lange M, Tönjes M, Dunkel I, Sperling SR. The cardiac transcription network modulated by Gata4, Mef2a, Nkx2.5, Srf, histone modifications, and microRNAs. *PLoS Genet* 2011; **7**: e1001313 [PMID: 21379568 DOI: 10.1371/journal.pgen.1001313]

24 **Takeuchi JK**, Bruneau BG. Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. *Nature* 2009; **459**: 708-711 [PMID: 19396158 DOI: 10.1038/nature08039]

25 **Dixon JE**, Dick E, Rajamohan D, Shakesheff KM, Denning C. Directed differentiation of human embryonic stem cells to interrogate the cardiac gene regulatory network. *Mol Ther* 2011; **19**: 1695-1703 [PMID: 21694703 DOI: 10.1038/mt.2011.125]

26 **Hao C**, Lu Z, Zhao Y, Chen Z, Shen C, Ma G, Chen L. Overexpression of GATA4 enhances the antiapoptotic effect of exosomes secreted from cardiac colony-forming unit fibroblasts *via* miRNA221-mediated targeting of the PTEN/PI3K/AKT signaling pathway. *Stem Cell Res Ther* 2020; **11**: 251 [PMID: 32586406 DOI: 10.1186/s13287-020-01759-8]

27 **Vanpoucke G**, Goossens S, De Craene B, Gilbert B, van Roy F, Berx G. GATA-4 and MEF2C transcription factors control the tissue-specific expression of the alphaT-catenin gene CTNNA3. *Nucleic Acids Res* 2004; **32**: 4155-4165 [PMID: 15302915 DOI: 10.1093/nar/gkh727]

28 **Materna SC**, Sinha T, Barnes RM, Lammerts van Bueren K, Black BL. Cardiovascular development and survival require Mef2c function in the myocardial but not the endothelial lineage. *Dev Biol* 2019; **445**: 170-177 [PMID: 30521808 DOI: 10.1016/j.ydbio.2018.12.002]

29 **Beeravolu N**, Brougham J, Khan I, McKee C, Perez-Cruet M, Chaudhry GR. Human umbilical cord derivatives regenerate intervertebral disc. *J Tissue Eng Regen Med* 2018; **12**: e579-e591 [PMID: 27690334 DOI: 10.1002/term.2330]

30 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]

31 **Ali SR**, Ahmad W, Naeem N, Salim A, Khan I. Small molecule 2'-deoxycytidine differentiates human umbilical cord-derived MSCs into cardiac progenitors in vitro and their in vivo xeno-transplantation improves cardiac function. *Mol Cell Biochem* 2020; **470**: 99-113 [PMID: 32415417 DOI: 10.1007/s11010-020-03750-6]

32 **Naeem N**, Haneef K, Kabir N, Iqbal H, Jamall S, Salim A. DNA methylation inhibitors, 5-azacytidine and zebularine potentiate the transdifferentiation of rat bone marrow mesenchymal stem cells into cardiomyocytes. *Cardiovasc Ther* 2013; **31**: 201-209 [PMID: 22954287 DOI: 10.1111/j.1755-5922.2012.00320.x]

33 **Peterkin T**, Gibson A, Loose M, Patient R. The roles of GATA-4,-5 and-6 in vertebrate heart development. *Semin Cell Dev Biol* 2005; **16**: 83-94 [PMID: 15659343 DOI: 10.1016/j.semcdb.2004.10.003]

34 **Kolodziejczyk SM**, Wang L, Balazsi K, DeRepentigny Y, Kothary R, Megeney LA. MEF2 is upregulated during cardiac hypertrophy and is required for normal post-natal growth of the myocardium. *Curr Biol* 1999; **9**: 1203-1206 [PMID: 10531040 DOI: 10.1016/S0960-9822(00)80027-5]

35 **Li H**, Zuo S, He Z, Yang Y, Pasha Z, Wang Y, Xu M. Paracrine factors released by GATA-4 overexpressed mesenchymal stem cells increase angiogenesis and cell survival. *Am J Physiol Heart Circ Physiol* 2010; **299**: H1772-H1781 [PMID:

20870802 DOI: 10.1152/ajpheart.00557.2010]

36 **Armiñán A**, Gandía C, García-Verdugo JM, Lledó E, Mullor JL, Montero JA, Sepúlveda P. Cardiac transcription factors driven lineage-specification of adult stem cells. *J Cardiovasc Transl Res* 2010; **3**: 61-65 [PMID: 20560034 DOI: 10.1007/s12265-009-9144-3]

37 **Yamada Y**, Sakurada K, Takeda Y, Gojo S, Umezawa A. Single-cell-derived mesenchymal stem cells overexpressing Csx/Nkx2.5 and GATA4 undergo the stochastic cardiomyogenic fate and behave like transient amplifying cells. *Exp Cell Res* 2007; **313**: 698-706 [PMID: 17208226 DOI: 10.1016/j.yexcr.2006.11.012]

38 **Fu JD**, Stone NR, Liu L, Spencer CI, Qian L, Hayashi Y, Delgado-Olguin P, Ding S, Bruneau BG, Srivastava D. Direct reprogramming of human fibroblasts toward a cardiomyocyte-like state. *Stem Cell Reports* 2013; **1**: 235-247 [PMID: 24319660 DOI: 10.1016/j.stemcr.2013.07.005]

39 **Serpooshan V**, Liu YH, Buikema JW, Galdos FX, Chirikian O, Paige S, Venkatraman S, Kumar A, Rawnsley DR, Huang X, Pijnappels DA, Wu SM. Nkx2.5+ Cardiomyoblasts Contribute to Cardiomyogenesis in the Neonatal Heart. *Sci Rep* 2017; **7**: 12590 [PMID: 28974782 DOI: 10.1038/s41598-017-12869-4]

40 **Kinnunen S**, Välimäki M, Tölli M, Wohlfahrt G, Darwich R, Komati H, Nemer M, Ruskoaho H. Nuclear Receptor-Like Structure and Interaction of Congenital Heart Disease-Associated Factors GATA4 and NKX2-5. *PLoS One* 2015; **10**: e0144145 [PMID: 26642209 DOI: 10.1371/journal.pone.0144145]

41 **Palmer S**, Groves N, Schindeler A, Yeoh T, Biben C, Wang CC, Sparrow DB, Barnett L, Jenkins NA, Copeland NG, Koentgen F, Mohun T, Harvey RP. The small muscle-specific protein Csl modifies cell shape and promotes myocyte fusion in an insulin-like growth factor 1-dependent manner. *J Cell Biol* 2001; **153**: 985-998 [PMID: 11381084 DOI: 10.1083/jcb.153.5.985]

42 **Clause KC**, Tchao J, Powell MC, Liu LJ, Huard J, Keller BB, Tobita K. Developing cardiac and skeletal muscle share fast-skeletal myosin heavy chain and cardiac troponin-I expression. *PLoS One* 2012; **7**: e40725 [PMID: 22808244 DOI: 10.1371/journal.pone.0040725]

43 **Stoppel WL**, Kaplan DL, Black LD 3rd. Electrical and mechanical stimulation of cardiac cells and tissue constructs. *Adv Drug Deliv Rev* 2016; **96**: 135-155 [PMID: 26232525 DOI: 10.1016/j.addr.2015.07.009]

44 **Scuderi GJ**, Butcher J. Naturally Engineered Maturation of Cardiomyocytes. *Front Cell Dev Biol* 2017; **5**: 50 [PMID: 28529939 DOI: 10.3389/fcell.2017.00050]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2022 September 26; 14(9): 714-728

DOI: 10.4252/wjsc.v14.i9.714

ISSN 1948-0210 (online)

ORIGINAL ARTICLE

Basic Study

Long non-coding RNA *SNHG16* promotes human placenta-derived mesenchymal stem cell proliferation capacity through the PI3K/AKT pathway under hypoxia

Xu-Dong Feng, Jia-Hang Zhou, Jun-Yao Chen, Bing Feng, Rui-Tian Hu, Jian Wu, Qiao-Ling Pan, Jin-Feng Yang, Jiong Yu, Hong-Cui Cao

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Jabbarpour Z, Iran; Luo Y, China; Wang J, China

Received: May 2, 2022
Peer-review started: May 2, 2022
First decision: June 11, 2022
Revised: June 24, 2022
Accepted: August 15, 2022
Article in press: August 15, 2022
Published online: September 26, 2022

Xu-Dong Feng, Jia-Hang Zhou, Jun-Yao Chen, Bing Feng, Jian Wu, Qiao-Ling Pan, Jin-Feng Yang, Jiong Yu, Hong-Cui Cao, State Key Laboratory for The Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, Zhejiang Province, China

Rui-Tian Hu, Department of Chemistry, Duke University, Durham, NC 27708, United States

Jian Wu, Jinan Microecological Biomedicine Shandong Laboratory, Jinan 250117, Shandong Province, China

Hong-Cui Cao, Key Laboratory of Diagnosis and Treatment of Aging and Physic-chemical Injury Diseases of Zhejiang Province, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, Zhejiang Province, China

Corresponding author: Hong-Cui Cao, Professor, State Key Laboratory for The Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, No. 79 Qingchun Road, Hangzhou 310003, Zhejiang Province, China. hccao@zju.edu.cn

Abstract

BACKGROUND

The effect of hypoxia on mesenchymal stem cells (MSCs) is an emerging topic in MSC biology. Although long non-coding RNAs (lncRNAs) and messenger RNAs (mRNAs) are reported to play a critical role in regulating the biological characteristics of MSCs, their specific expression and co-expression profiles in human placenta-derived MSCs (hP-MSCs) under hypoxia and the underlying mechanisms of lncRNAs in hP-MSC biology are unknown.

AIM

To reveal the specific expression profiles of lncRNAs in hP-MSCs under hypoxia and initially explored the possible mechanism of lncRNAs on hP-MSC biology.

METHODS

Here, we used a multigas incubator (92.5% N_2, 5% CO_2, and 2.5% O_2) to mimic the

hypoxia condition and observed that hypoxic culture significantly promoted the proliferation potential of hP-MSCs. RNA sequencing technology was applied to identify the exact expression profiles of lncRNAs and mRNAs under hypoxia.

RESULTS

We identified 289 differentially expressed lncRNAs and 240 differentially expressed mRNAs between the hypoxia and normoxia groups. Among them, the lncRNA *SNHG16* was upregulated under hypoxia, which was also validated by reverse transcription-polymerase chain reaction. *SNHG16* was confirmed to affect hP-MSC proliferation rates using a *SNHG16* knockdown model. *SNHG16* overexpression could significantly enhance the proliferation capacity of hP-MSCs, activate the PI3K/AKT pathway, and upregulate the expression of cell cycle-related proteins.

CONCLUSION

Our results revealed the specific expression characteristics of lncRNAs and mRNAs in hypoxia-cultured hP-MSCs and that lncRNA *SNHG16* can promote hP-MSC proliferation through the PI3K/AKT pathway.

Key Words: Human placenta-derived mesenchymal stem cell; Hypoxia; Long non-coding RNAs; Proliferation; Mesenchymal stem cell

Core Tip: This study revealed the specific expression and co-expressed profiles of long non-coding RNAs and messenger RNAs in human placenta-derived mesenchymal stem cells under hypoxia by RNA sequencing assays. Through the performance of a series of systemic bioinformatic analyses, the hypoxia-responsive long non-coding RNA *SNHG16* that may play a role in proliferation was screened out. Furthermore, through the use of molecular biology experiments, *SNHG16* was found to affect human placenta-derived mesenchymal stem cell proliferation rates and cell cycle progression by activating the PI3K/AKT pathway and upregulating the expression of the key cell cycle regulators.

Citation: Feng XD, Zhou JH, Chen JY, Feng B, Hu RT, Wu J, Pan QL, Yang JF, Yu J, Cao HC. Long non-coding RNA *SNHG16* promotes human placenta-derived mesenchymal stem cell proliferation capacity through the PI3K/AKT pathway under hypoxia. *World J Stem Cells* 2022; 14(9): 714-728
URL: https://www.wjgnet.com/1948-0210/full/v14/i9/714.htm
DOI: https://dx.doi.org/10.4252/wjsc.v14.i9.714

INTRODUCTION

Recently, mesenchymal stem cells (MSCs) have gained much attention due to their therapeutic effects and potential applications in regenerative medicine[1]. MSCs have recently been shown to have therapeutic efficacy in various disease models and clinical diseases such as liver injury, coronavirus disease 2019, and Crohn's disease[2-5]. MSCs have been reported to be present in bone marrow, placenta, umbilical cord, and adipose tissue and can be efficiently isolated[6-8]. However, the application of MSCs is limited due to the difficulty in obtaining the large numbers of MSCs required for clinical treatment (3×10^7 cells *per* infusion)[9]. Tissue-derived primary MSCs occur in small numbers and require *in vitro* expansion before transplantation. Human placenta-derived MSCs (hP-MSCs) are a ubiquitous type of MSCs with lower immunogenicity and higher proliferative potential compared to bone marrow-derived MSCs, but these advantages may be compromised by inappropriate culture or changes in the *in vitro* environment.

To improve the proliferation potential of hP-MSCs *in vitro*, most researchers use different methods to stimulate the microenvironment of MSCs *in vivo*[10]. Among the proposed approaches to mimic the natural cellular microenvironment, hypoxia has garnered enormous interest. Hypoxia has been observed in different tissue niches, including the placenta (1%-5% O_2) where hP-MSCs reside[11]. Since the oxygen concentration (almost 21%) in the *ex vivo* culture system is much higher than the physiological oxygen concentration in the body, hypoxia could act as a physiological stimulus with a significant influence on cell fate. Numerous studies have reported that hypoxia can affect various biological properties of MSCs, such as proliferation capacity, multidirectional differentiation potential, migration, and apoptosis[12-14]. However, the underlying molecular mechanisms by which hypoxia regulates MSC biology remain unclear.

Long non-coding RNAs (lncRNAs) are RNAs longer than 200 nt with no protein-coding potential [15]. LncRNAs are the coordinators of the cellular biological regulatory network, participating in a variety of biological and pathological cellular processes such as cellular survival, proliferation, or migration through regulation of gene expression at transcriptional, post-transcriptional, or translational levels[16]. With advancements in gene sequencing technology, more and more lncRNAs related to cellular functions have been identified. However, the impact of hypoxia on the lncRNA expression profile of MSCs remains unclear. In addition, the roles of hypoxia-responsive lncRNAs remain to be explored. In this study, we investigated the effect of hypoxia on the proliferation potential of hP-MSCs and explored the role of lncRNAs in it.

MATERIALS AND METHODS

Cell culture

The protocols for hP-MSC isolation and hypoxic culture were as previously described[7]. All protocols for the processing of human tissues and cells were approved by the Ethics Committee of The First Affiliated Hospital of Zhejiang University (No. 2020-1088).

Colony-forming unit-fibroblast assay

For the colony-forming unit-fibroblast assay, 1000 hP-MSCs were plated on six-well plates in triplicate and cultured in complete medium for 14 d under normoxic or hypoxic conditions with medium changes every 3 d. Colonies were fixed with paraformaldehyde and then stained with crystal violet for enumeration.

Cell counting kit-8 assay

The corresponding cells were inoculated into 96-well cell culture plates at a density of 2000 cells *per* well. After 24, 48, 72 or 96-h culture, 10 μL of cell counting kit-8 reagent (Dojindo, Kumamoto, Japan) was added into each well to incubate for 2 h. The optical density value at 450 nm was measured using a microplate reader.

Flow cytometry analysis of cell cycle

The cells were collected with trypsin and fixed with cold 70% ethanol for 2 h. Fixed cells were then treated with propidium iodide staining solution (Beyotime, Nanjing, China). The cells were finally analyzed by flow cytometry. The proportions of cell population in G0/G1, S, and G2/M phases of the cell cycle were fitted and calculated using ModFit software.

Quantitative real-time polymerase chain reaction

Total RNA of cells was obtained using Trizol reagent (Invitrogen, Carlsbad, CA, United States) following the manufacturer's protocol; the concentration of total RNA was quantified using a NanoDrop-2000 (Thermo Fisher Scientific, Waltham, MA, United States). cDNA was synthesized by reverse transcription reaction using a commercial lncRNA quantitative reverse transcription polymerase chain reaction (PCR) Starter Kit (RiboBio, Guangzhou, China). The final relative expression levels of genes were analyzed through the $2^{-\Delta\Delta Ct}$ method using *GAPDH* as the internal control. Primers were as follows: *GAPDH*: (forward) 5′-ACAACTTTGGTATCGTGGAAGG-3′, (reverse) 5′-GCCATCACGC-CACAGTTTC-3′; *SNHG16*: (forward) 5′-GTTGCCACCCACAACCATT-3′, and (reverse) 5′-GCGGAGACACCAGGAGAACT-3′.

Western blot assay

The cellular protein was harvested using RIPA lysis buffer supplemented with protease and phosphatase inhibitor cocktail (Beyotime). The protein concentrations were detected using a BCA kit (Beyotime). The western blot was conducted as previously described[7]. The primary antibodies were anti-β-actin (Abcam, Cambridge, United Kingdom), anti-GAPDH (Abcam), anti-hypoxia-inducible factor 1α (HIF-1α) (Cell Signaling Technology, Danvers, MA, United States), anti-c-MYC (Abcam), anti-proliferating cell nuclear antigen (Abcam), anti-CDK2 (Abcam), anti-CDK4 (Abcam), anti-CDK6 (Abcam), anti-CyclinD1 (Abcam), anti-CyclinE1 (Abcam), anti-AKT (Abcam), and anti-phospho-AKT (Abcam).

RNA sequencing

Whole-transcriptome sequencing was quantitatively analyzed by Oebiotech (Shanghai, China). The libraries [including lncRNA and messenger RNA (mRNA)] were generated using TruSeq Stranded Total RNA with Ribo-Zero Gold (Illumina, San Diego, CA, United States) according to the manual. RNA was then sequenced on a HiSeq 2500 instrument (Illumina). Differential expression analysis of lncRNA and mRNA between the hypoxic and normoxic groups was conducted using the DESeq software package. The differentially expressed genes were identified with the criteria of fold change > 1.5 and $P < 0.05$. The

Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed for differentially expressed mRNAs to explore their biological functions. Association analysis between lncRNAs and transcription factors and lncRNA–mRNA co-expression analysis were conducted to investigate lncRNA functions in cell biology.

Cell transfection

Lentivirus-mediated short hairpin RNA for silencing *SNHG16* in cells and recombinant lentivirus for *SNHG16* overexpression were constructed by Genomeditech (Shanghai, China). Transfection was performed following the manufacturer's instructions. Lentiviruses were added to infect cells at a multiplicity of infection of 50:1.

Statistical analysis

All data were expressed as the mean ± SD. Statistical evaluation of two groups was conducted using Student's *t* test; a P value < 0.05 was considered to indicate statistical significance.

RESULTS

Hypoxic culture facilitated hP-MSC proliferation

HIF-1α is a critical regulator of cellular adaptation to the hypoxic microenvironment. When the expression of HIF-1α protein under hypoxia was assessed by western blot, hypoxia induced the expression of HIF-1α in hP-MSCs and stabilized its expression level during cell growth (Figure 1A). In addition, hP-MSCs cultured under hypoxia appeared to be relatively small, with a spindle-shaped morphology (Figure 1B). The cell counting kit-8 assay showed that hP-MSCs had higher proliferation potential (P < 0.0001) when they were maintained under hypoxia (Figure 1C). Similarly, the colony-forming unit-fibroblast assay indicated that hypoxia enhanced the hP-MSC proliferation rate. Although the difference in the number of hP-MSC colonies between the hypoxia and normoxia groups was not significant (P = 0.249), the colony size of the hypoxia group was larger with darker staining, indicating a higher number of cells (Figure 1D). c-MYC and proliferating cell nuclear antigen are molecules closely related to cell proliferation and can be adopted to determine the status of cell proliferation. As expected, hypoxia significantly increased the expression of c-MYC and proliferating cell nuclear antigen, indicating that cells proliferated more rapidly under hypoxia (Figure 1E).

Hypoxia specifically altered the lncRNA and mRNA expression profiles of hP-MSCs

To further investigate the influence of hypoxia on hP-MSCs, whole-transcriptome sequencing was performed. First, six high-throughput sequenced transcriptomes were generated, containing over 650 million clean reads, among which three were from the normoxic group and three were from the hypoxic group. More than 96% of the raw reads were high-quality clean reads (Table 1). Ultimately, 10387 putative lncRNAs and 16041 mRNAs were identified. We further identified 289 differentially expressed lncRNAs (135 upregulated and 154 downregulated) and 240 differentially expressed mRNAs (156 upregulated and 84 downregulated) in the hypoxia group compared to normoxia group (Figure 2). Heatmap analysis clearly distinguished the hP-MSCs cultured under hypoxia from those cultured under normoxia. The top 20 differentially expressed lncRNAs and mRNAs are summarized in Tables 2 and 3.

Differentially expressed mRNAs participated in cell proliferation function

The top ten enriched GO terms in biological process, molecular function, and cellular component were determined. For biological process, the differentially expressed mRNAs were related to regulation of cell growth (GO: 0001558), positive regulation of MAP kinase activity (GO: 0043406), and response to hypoxia (GO: 0001666) (Figure 3A). KEGG pathway enrichment analysis revealed several significantly enriched pathways, such as the HIF-1 signaling pathway (KEGG: hsa04066), Jak-STAT signaling pathway (KEGG: hsa04630), and Rap1 signaling pathway (KEGG: has04015) (Figure 3B). We further performed GO and KEGG analyses on the upregulated and downregulated genes separately. Upregulated genes were involved in regulation of cell growth (GO: 0001558) and regulation of cell proliferation (GO: 0042127) (Figure 3C) and were enriched in the HIF-1 signaling pathway (KEGG: hsa04066), Jak-STAT signaling pathway (KEGG: hsa04630), and AMPK signaling pathway (KEGG: hsa04152) (Figure 3D). Thus, hypoxia mainly affected cell functions, such as proliferation, by upregulating the expression of certain genes through several signaling pathways.

SNHG16 was a potential promotor of hP-MSC proliferation ability

Hypoxia affected cell proliferation by regulating cell cycle progression as the percentages of S (P = 0.011) and G2/M phase cells (P = 0.014) were larger under the hypoxic condition (Figure 4A). At the same time, the PI3K/AKT pathway was activated under hypoxia (Figure 4B). The PI3K/AKT pathway is responsible for coordinating a diverse range of cell functions, including proliferation and survival. These findings suggest that hypoxia can activate the PI3K/AKT pathway and modulate the cell cycle.

Table 1 Summary of the RNA sequencing data

Summary	MSC_N1	MSC_N2	MSC_N3	MSC_H1	MSC_H2	MSC_H3
Raw reads (M)	103.22	113.01	117.09	114.00	117.77	106.81
Clean reads (M)	99.95	109.86	113.73	110.04	113.72	103.86
Clean reads rate (%)	96.83	97.21	97.13	96.53	96.56	97.24
Q30 (%)	94.32	94.63	94.62	94.22	94.18	94.78
GC (%)	46.71	45.65	45.35	46.18	44.99	45.92
Total mapped reads	320502139			324502629		
Uniquely mapped reads	308534583			312982527		

Q30 (%) represents the proportion of the data in which the quality values are > Q30 in the raw data; MSC_N: Human placenta-derived mesenchymal stem cells cultured under normoxic condition; MSC_H: Human placenta-derived mesenchymal stem cells cultured under hypoxic condition.

Table 2 Summary of the top 20 differentially expressed long non-coding RNAs

Upregulated lncRNA	Fold-change	Downregulated lncRNA	Fold-change
ENST00000480904	144.7046633	TCONS_00024987	558.6529515
ENST00000420168	88.58973499	TCONS_00022901	305.9141231
ENST00000447687	51.87793204	ENST00000580684	101.5147448
TCONS_00022897	43.87602707	XR_951092.3	96.15131381
NR_135828.1	43.01226344	TCONS_00040744	85.07807992
ENST00000652331	37.64067361	NR_151707.1	75.91477831
ENST00000615566	35.57471309	XR_924538.2	51.52143558
XR_001740831.1	30.29369126	ENST00000533146	47.14143364
XR_943245.2	29.4766044	NR_046472.1	45.2199291
ENST00000641463	29.12107436	ENST00000379848	34.42041339
ENST00000587838	28.77216197	XR_002957073.1	33.6627267
NR_027295.2	28.51478291	NR_138037.1	31.71645024
XR_001738493.2	26.08189868	ENST00000424751	31.28206241
ENST00000622955	24.98325918	ENST00000476224	29.12601955
ENST00000437589	23.11958922	NR_152515.1	28.62941025
NR_028397.1	22.10295425	ENST00000608741	25.94241325
NR_138259.1	21.16258012	XR_001740695.2	25.76946681
XR_947992.2	21.13351381	NR_102280.1	25.69150571
XR_930796.2	18.16731229	ENST00000513626	23.42886779
ENST00000542086	17.05330496	NR_132369.1	21.10943719

lncRNA: Long non-coding RNA.

To explore whether there are specific hypoxia-responsive lncRNAs that play a role in hypoxia-promoted cell proliferation, association analysis between lncRNAs and transcription factors and lncRNA–mRNA co-expression analysis were performed. *SNHG16* was related to the expression of *PIK3R5*, a gene encoding the regulatory subunit of the PI3K gamma complex (Figure 4C and D). *SNHG16* was also correlated with *FOSB*, a key transcription factor in the cell cycle (Figure 4E). Quantitative reverse transcription PCR analysis confirmed that hypoxia induced the expression of *SNHG16* (P = 0.003), consistent with the results of RNA sequencing (Figure 4F). Thus, *SNHG16* is a potential promoter of hP-MSC proliferation under hypoxia.

Table 3 Summary of the top 20 differentially expressed messenger RNAs

Upregulated mRNA	Fold-change	Downregulated mRNA	Fold-change
S100A1	32.28708068	*MMP13*	32.7146796
IL20	31.01976446	*RASAL3*	25.22521191
GUCY2D	18.95647724	*ITGAM*	21.2376519
PIK3R5	16.27652505	*FGF14*	13.88641698
KCNJ15	9.485618545	*KCNS2*	9.402226087
CA9	9.092268285	*DCT*	7.332486828
AK4	8.577190243	*TMEM247*	6.224926429
CD99	7.853653254	*TNFRSF4*	5.89336975
VSIG2	6.545400641	*SOX7*	5.002461264
CKMT2	6.072884506	*TXNIP*	3.914446648
FOLR1	5.195878685	*NBPF6*	3.514713231
C5orf46	5.180793591	*PDF*	3.476083157
PPFIA4	4.979164209	*LHX4*	3.46662373
TEC	4.732591252	*CD14*	3.005111835
INHBB	4.597149928	*HHIPL2*	2.918560997
GLDC	4.140246682	*RASL11B*	2.770760137
GPR146	4.107008037	*LMO3*	2.767289903
VASH2	4.051761622	*MYH11*	2.728808288
C4orf47	3.969880654	*DIRAS2*	2.718905726
SCHIP1	3.945719906	*AJM1*	2.696612138

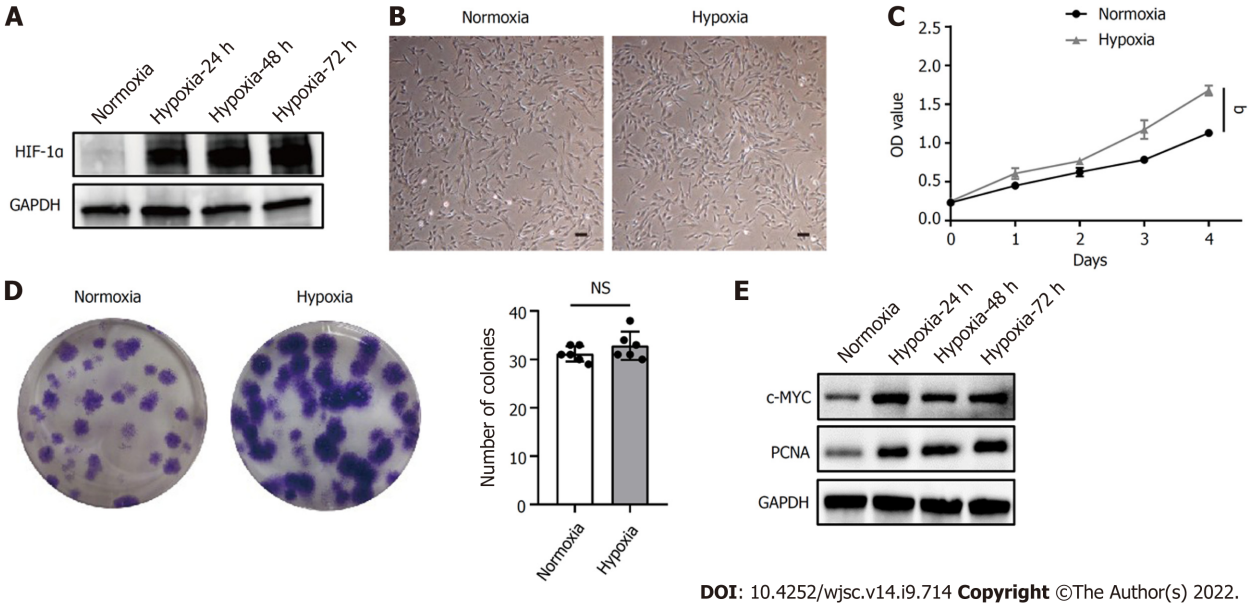
mRNA: Messenger RNA.

SNHG16 promoted proliferation of hP-MSCs via the PI3K/AKT pathway

To further confirm the biological function of *SNHG16* in hP-MSCs, short hairpin RNA was used to specifically knock down *SNHG16*, whereas lentivirus overexpressing *SNHG16* was used to increase *SNHG16* expression. By transfecting *SNHG16* short hairpin RNA, we found that sh-*SNHG16* significantly downregulated *SNHG16* expression ($P < 0.0001$) by up to 80% (Figure 5A). The cell counting kit-8 assay then revealed that depletion of *SNHG16* could attenuate the proliferation ability of hP-MSCs under both normoxia ($P = 0.0003$) and hypoxia ($P = 0.0007$) (Figure 5B and Supplementary Figure 1). Moreover, *SNHG16* knockdown decreased the cell numbers in S phase ($P = 0.022$) and increased the ratio of cells in the G0/G1 phase ($P = 0.003$) (Figure 5C). Furthermore, western blot showed that knockdown of *SNHG16* downregulated the phosphorylation of AKT and the expression of several important cell cycle regulators, including CDK2, CDK4, CDK6, cyclin E1, and cyclin D1 (Figure 5D). Subsequently, we evaluated the effect of *SNHG16* overexpression on cell proliferation and cell cycle transition. Quantitative reverse transcription PCR indicated that *SNHG16* was upregulated approximately 8-fold ($P = 0.0001$) when transfected with lentivirus overexpressing *SNHG16* (Figure 6A). Overexpression of *SNHG16* greatly enhanced the proliferative rate of hP-MSCs ($P < 0.0001$) and caused a mild increase in the ratio of cells in the S ($P = 0.027$) and G2/M phases ($P = 0.003$) (Figure 6B and C). The expression of G1 to S phase transition-related genes in the *SNHG16* overexpression group increased along with the activation of the PI3K/AKT pathway (Figure 6D). Overall, these data demonstrated that *SNHG16* could facilitate the growth and cell cycle transition of hP-MSCs through activating the PI3K/AKT pathway.

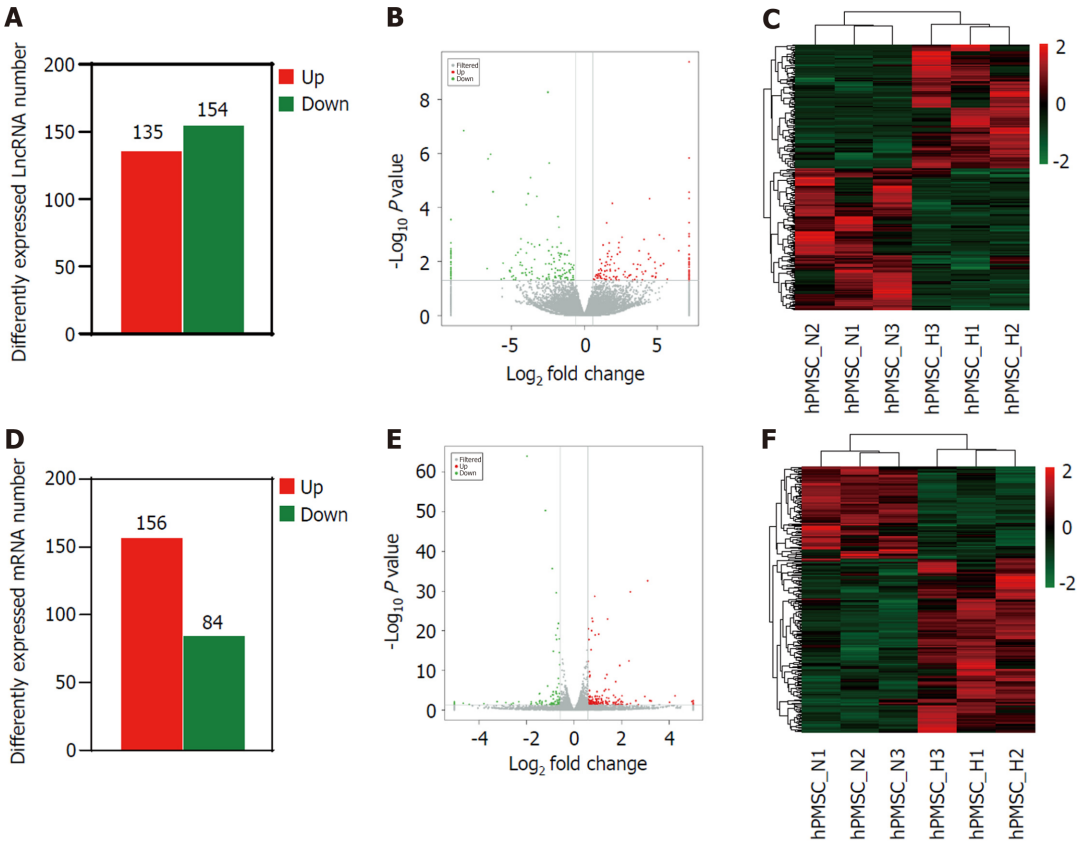
DISCUSSION

MSCs have great potential to cure a variety of diseases, as evidenced by the rapid growth in the number of published preclinical and clinical studies. However, MSCs are found in very small numbers in most adult tissues, such as bone marrow, placenta, adipose tissue, umbilical cord, amniotic fluid, and muscle



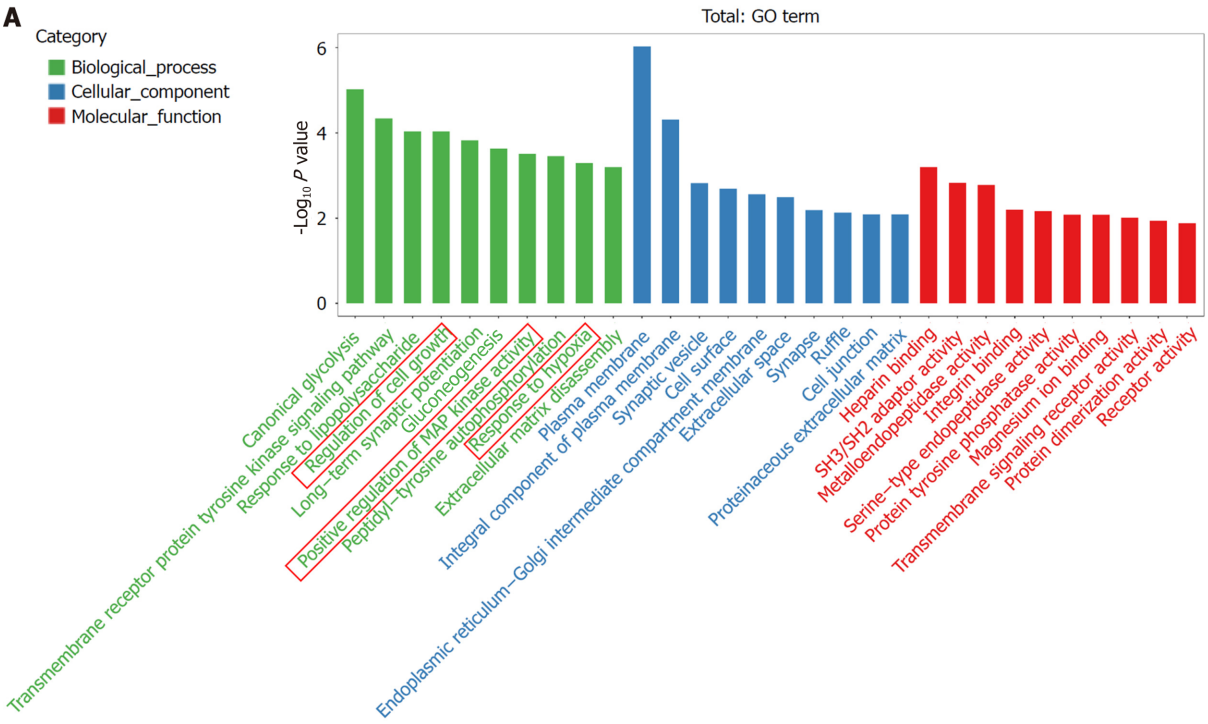
DOI: 10.4252/wjsc.v14.i9.714 **Copyright** ©The Author(s) 2022.

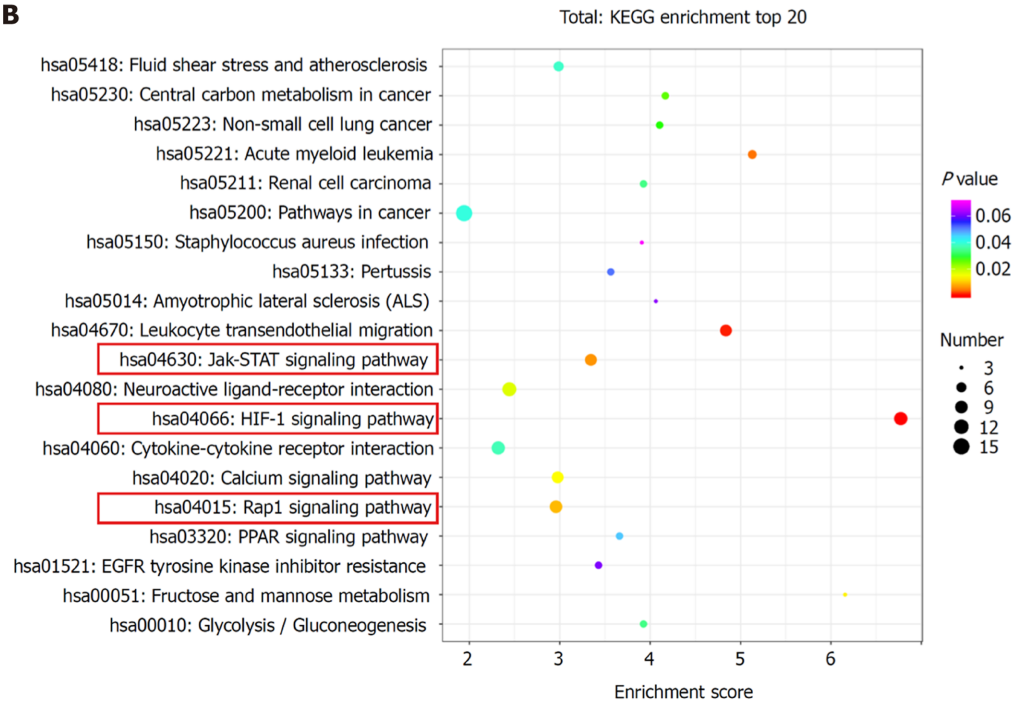
Figure 1 Hypoxia facilitated human placenta-derived mesenchymal stem cell growth and proliferation. A: Western blot analysis of hypoxia-inducible factor 1α expression in human placenta-derived mesenchymal stem cells (hP-MSCs) under hypoxic culture for 24, 48, or 72 h; B: Morphology of the cultured hP-MSCs under hypoxia (scale bars, 100 μm); C: Proliferation curves of hP-MSCs were established based on cumulative cell numbers at different incubation times (0, 1, 2, 3, and 4 d) under normoxia or hypoxia; D: Colony size and colony number of hP-MSCs under normoxic or hypoxic culture (n = 6); E: The protein expression of c-MYC and proliferating cell nuclear antigen in hP-MSCs under hypoxic culture for 24, 48, or 72 h. Data are presented as means ± SD. $^{a}P < 0.05$. NS: No significance; PCNA: Proliferating cell nuclear antigen; HIF-1α: Hypoxia-inducible factor 1α.

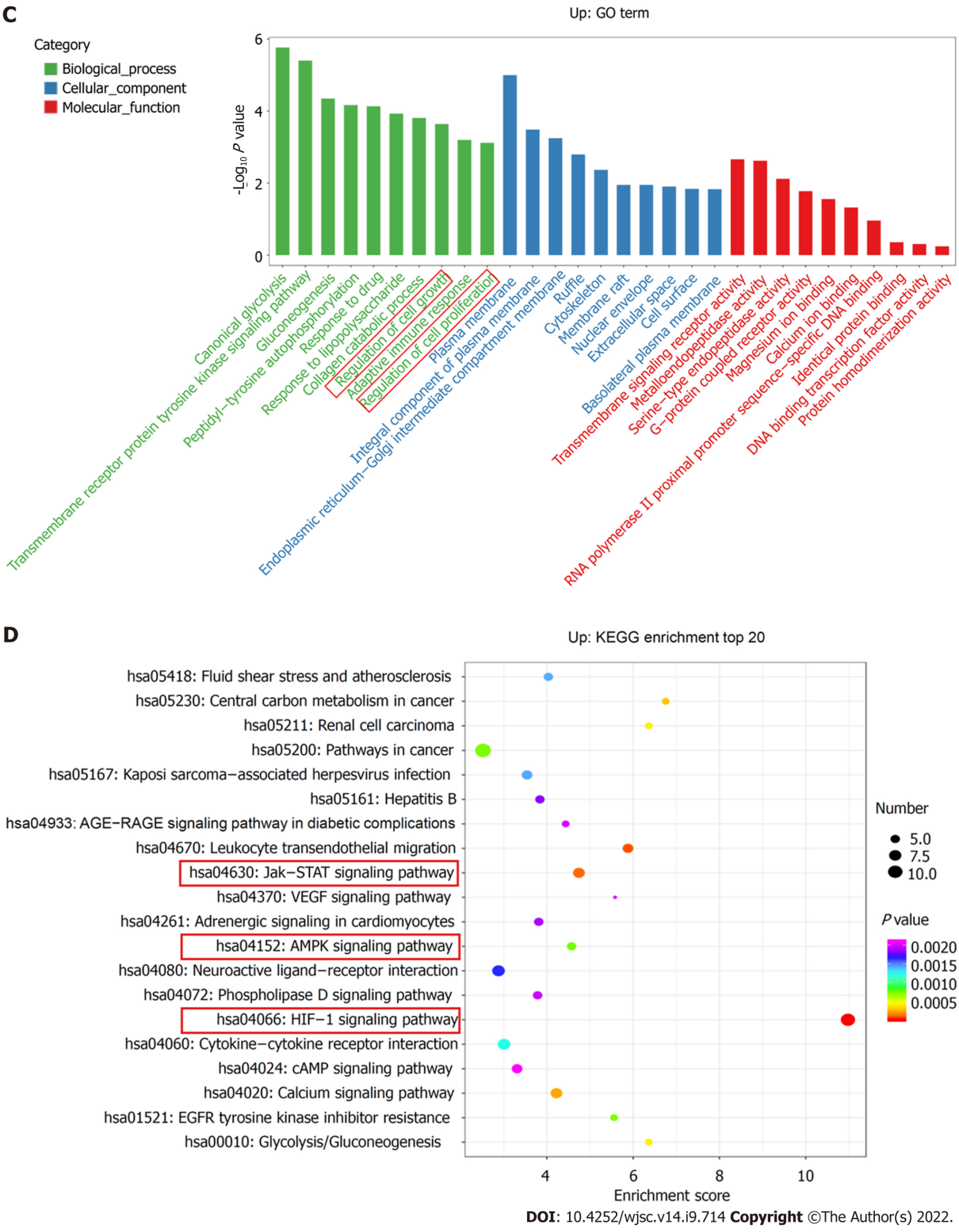


DOI: 10.4252/wjsc.v14.i9.714 **Copyright** ©The Author(s) 2022.

Figure 2 Long non-coding RNAs and messenger RNA expression profiles under hypoxia and normoxia. A: Number of differentially expressed long non-coding (lnc)RNAs between hypoxia and normoxia; B: Volcano plot depicting differentially expressed lncRNAs between hypoxia and normoxia; C: Heatmap of all differentially expressed lncRNAs identified in hypoxia *vs* normoxia; D: Number of differentially expressed messenger (m)RNAs; E: Volcano plot of differentially expressed mRNAs; F: Heatmap showing hierarchical clustering of differentially expressed mRNAs.


A
Category
Biological_process
Cellular_component
Molecular_function
Total: GO term
-Log10 P value
0
2
4
6
Canonical glycolysis
Transmembrane receptor protein tyrosine kinase signaling pathway
Response to lipopolysaccharide
Regulation of cell growth
Long−term synaptic potentiation
Gluconeogenesis
Positive regulation of MAP kinase activity
Peptidyl−tyrosine autophosphorylation
Response to hypoxia
Extracellular matrix disassembly
Plasma membrane
Integral component of plasma membrane
Endoplasmic reticulum−Golgi intermediate compartment membrane
Synaptic vesicle
Cell surface
Extracellular space
Synapse
Ruffle
Cell junction
Proteinaceous extracellular matrix
Heparin binding
SH3/SH2 adaptor activity
Metalloendopeptidase activity
Serine−type endopeptidase activity
Integrin binding
Protein tyrosine phosphatase activity
Magnesium ion binding
Transmembrane signaling receptor activity
Protein dimerization activity
Receptor activity


B
Total: KEGG enrichment top 20
hsa05418: Fluid shear stress and atherosclerosis
hsa05230: Central carbon metabolism in cancer
hsa05223: Non-small cell lung cancer
hsa05221: Acute myeloid leukemia
hsa05211: Renal cell carcinoma
hsa05200: Pathways in cancer
hsa05150: Staphylococcus aureus infection
hsa05133: Pertussis
hsa05014: Amyotrophic lateral sclerosis (ALS)
hsa04670: Leukocyte transendothelial migration
hsa04630: Jak-STAT signaling pathway
hsa04080: Neuroactive ligand-receptor interaction
hsa04066: HIF-1 signaling pathway
hsa04060: Cytokine-cytokine receptor interaction
hsa04020: Calcium signaling pathway
hsa04015: Rap1 signaling pathway
hsa03320: PPAR signaling pathway
hsa01521: EGFR tyrosine kinase inhibitor resistance
hsa00051: Fructose and mannose metabolism
hsa00010: Glycolysis / Gluconeogenesis
2
3
4
5
6
Enrichment score
P value
0.06
0.04
0.02
Number
3
6
9
12
15

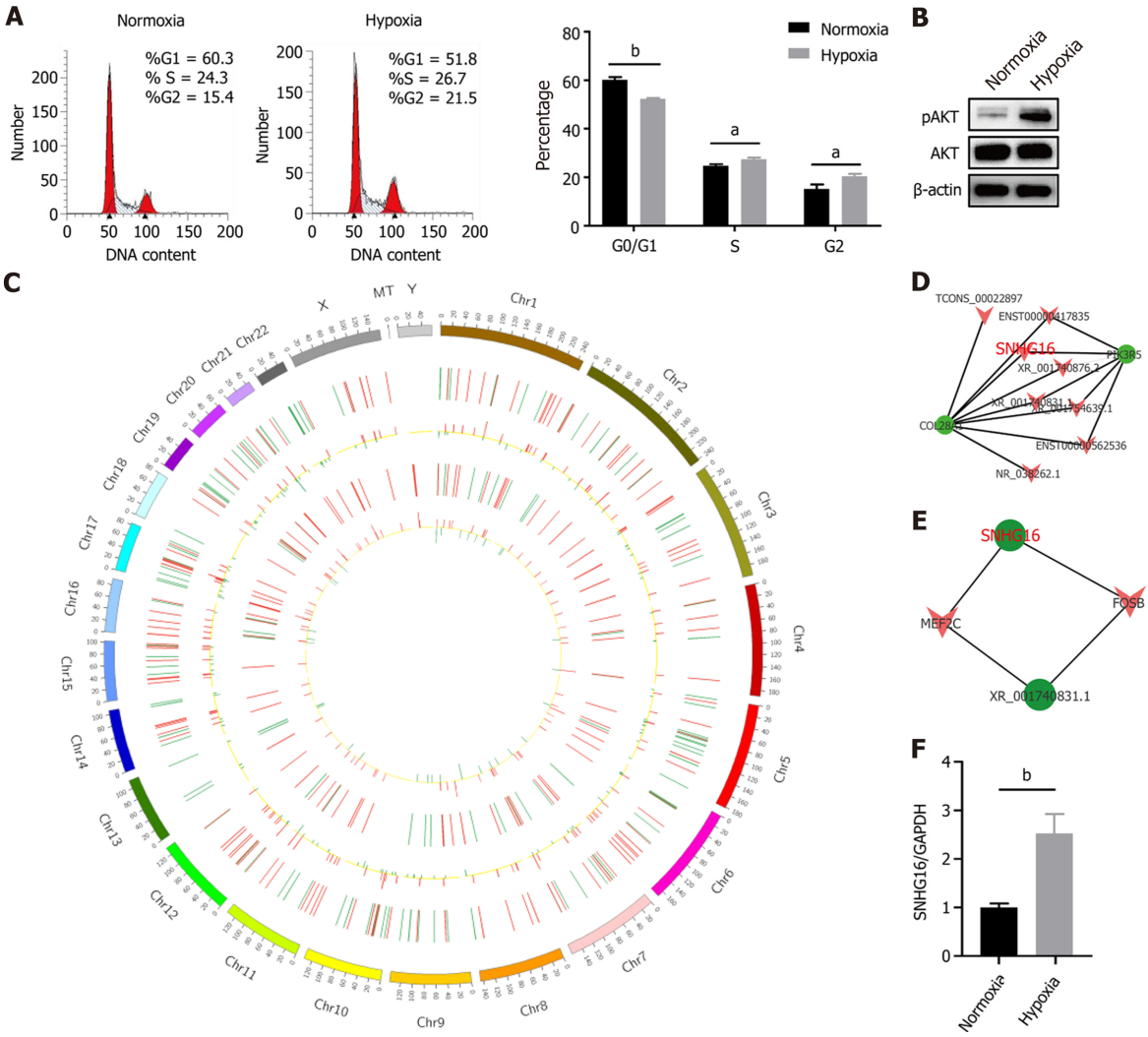


DOI: 10.4252/wjsc.v14.i9.714

Figure 3 Gene Ontology terms and Kyoto Encyclopedia of Genes and Genomes pathway analyses. A: Enrichment of biological process, cellular component, and molecular function in all differentially expressed messenger RNAs (mRNAs); B: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of all differentially expressed mRNAs; the top 20 are listed; C: Gene Ontology (GO) annotation and functional enrichment of upregulated mRNAs; D: KEGG pathway enrichment analysis of upregulated mRNAs.

[17,18]. To generate sufficient clinical therapeutic quantities, *in vitro* expansion is necessary[19]. Managing and modifying culture conditions during amplification of MSCs *in vitro* is critical for the manufacture of effective cell therapies, as these *in vitro* culture conditions affect the cell properties and cell behaviors after transplantation[20].

MSCs are widely located in the hypoxic microenvironment[21,22]. This physiological oxygen concentration is significantly lower than normoxic conditions typically used for MSC culture in the laboratory.



DOI: 10.4252/wjsc.v14.i9.714 **Copyright** ©The Author(s) 2022.

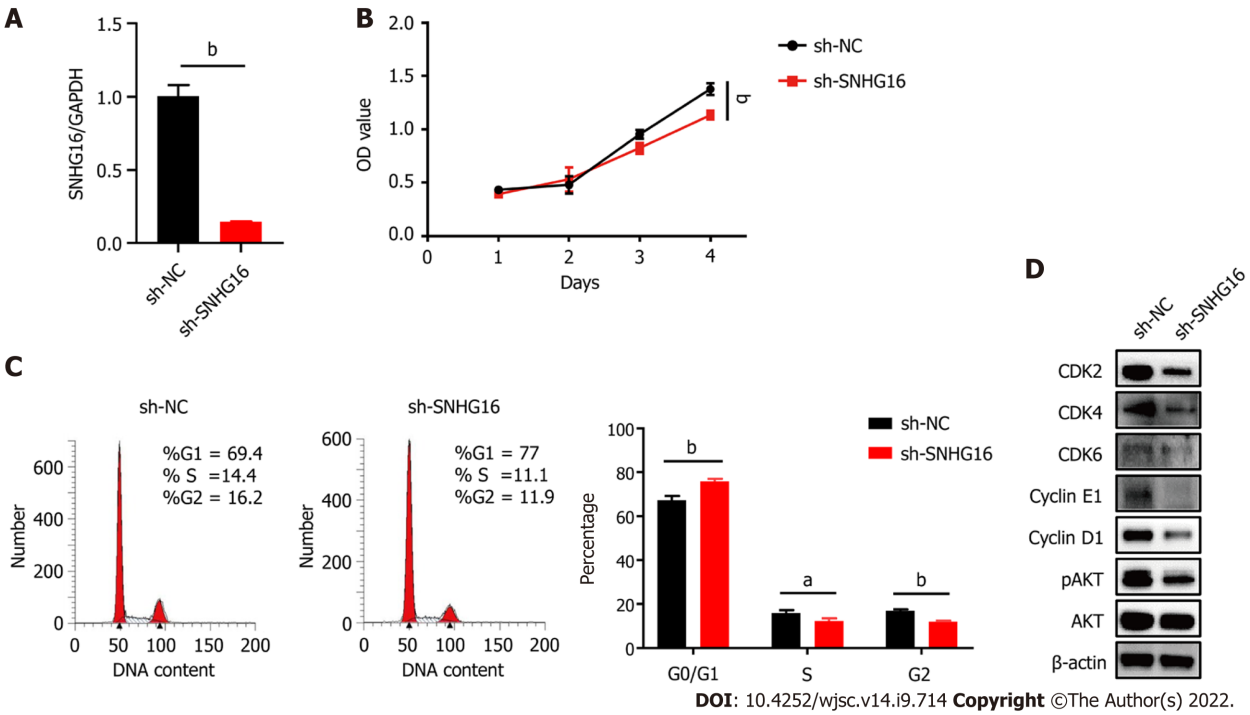
Figure 4 *SNHG16* was a potential promotor of human placenta-derived mesenchymal stem cell proliferation ability. A: Cell cycle analysis of human placenta-derived mesenchymal stem cells (hP-MSCs) under hypoxic culture *via* flow cytometry; B: Western blot analysis of AKT phosphorylation in hP-MSCs exposed to hypoxia; C: Circos plot of the long non-coding RNAs (lncRNAs)-messenger (m)RNA co-expression network. The outermost circle is the autosomal distribution. The second and third circles are the distribution of differentially expressed lncRNAs on chromosomes. The red line represents upregulation, and the green line represents downregulation. Higher bars indicate a greater number of differential genes in the interval. The fourth and fifth circles are the distribution of differentially expressed genes on chromosomes, with the same interpretation as lncRNA; D: Part of lncRNA-mRNA interaction network analysis visualized using the Cytoscape software; E: Part of the association analysis of differentially expressed lncRNAs and transcription factors; F: Effects of hypoxia on the expression of *SNHG16* in hP-MSCs by quantitative reverse transcription polymerase chain reaction. Data are presented as means ± standard deviation. $^{b}P < 0.01$.

Therefore, the application of physiological oxygen tension in stem cell research has attracted attention. Culturing MSCs under hypoxia has been consistently associated with increased cell proliferative rate, increased clonogenicity, decreased spontaneous differentiation, transcriptional alterations, and other cellular behaviors[11,23-25].

In the current study, we focused on the influence of hypoxia on hP-MSC proliferation ability. We found that hypoxic culture could facilitate hP-MSC proliferation, but enhanced clonogenicity under hypoxia was not observed in hP-MSCs. This finding provides a basis for exploring the underlying mechanism of the increased proliferation of hP-MSCs under hypoxic conditions.

Previous findings suggested that lncRNAs could exert regulatory function in MSC proliferation or differentiation. For example, Meng *et al*[26] revealed that *lincRNA-p21* promotes the migration and survival capabilities of mouse bone marrow-derived MSCs *via* the HIF-1α/CXCR4 and CXCR7 axis under hypoxia[26]. In addition, *LINC01119* negatively regulates osteogenic differentiation of human bone marrow-derived MSCs, while the lncRNAs *LOC100126784* and *POM121L9P* improve the osteogenic differentiation of human bone marrow-derived MSCs[27,28]. However, the role of lncRNAs in hP-MSCs has rarely been reported.

Here, we employed RNA sequencing technology to obtain a comprehensive and systematic understanding of lncRNAs in hP-MSCs under hypoxia condition. A total of 289 lncRNAs (135 upregulated and 154 downregulated) and 240 mRNAs (156 upregulated and 84 downregulated) were differentially



DOI: 10.4252/wjsc.v14.i9.714

Figure 5 Knockdown of *SNHG16* attenuated the proliferation ability of human placenta-derived mesenchymal stem cells. A: Quantitative reverse transcription polymerase chain reaction analysis of relative *SNHG16* expression after transfection of *SNHG16* short hairpin RNA (sh-*SNHG16*) and the corresponding controls (sh-NC) in human placenta-derived mesenchymal stem cells; B: Cell proliferation capacity evaluated by cell counting kit-8 assay; C: Cell cycle measured by flow cytometry; D: The G1 to S phase transition-related proteins and p-AKT detected by western blot analysis. Data are presented as means ± standard deviation. $^{b}P < 0.01$.

expressed between the hypoxia and normoxia groups. Expression profiles of these differentially expressed genes were clustered hierarchically. GO and KEGG analyses suggested that the most enriched genes were positioned in the plasma membrane and related to regulation of cell growth and HIF-1 signaling pathway.

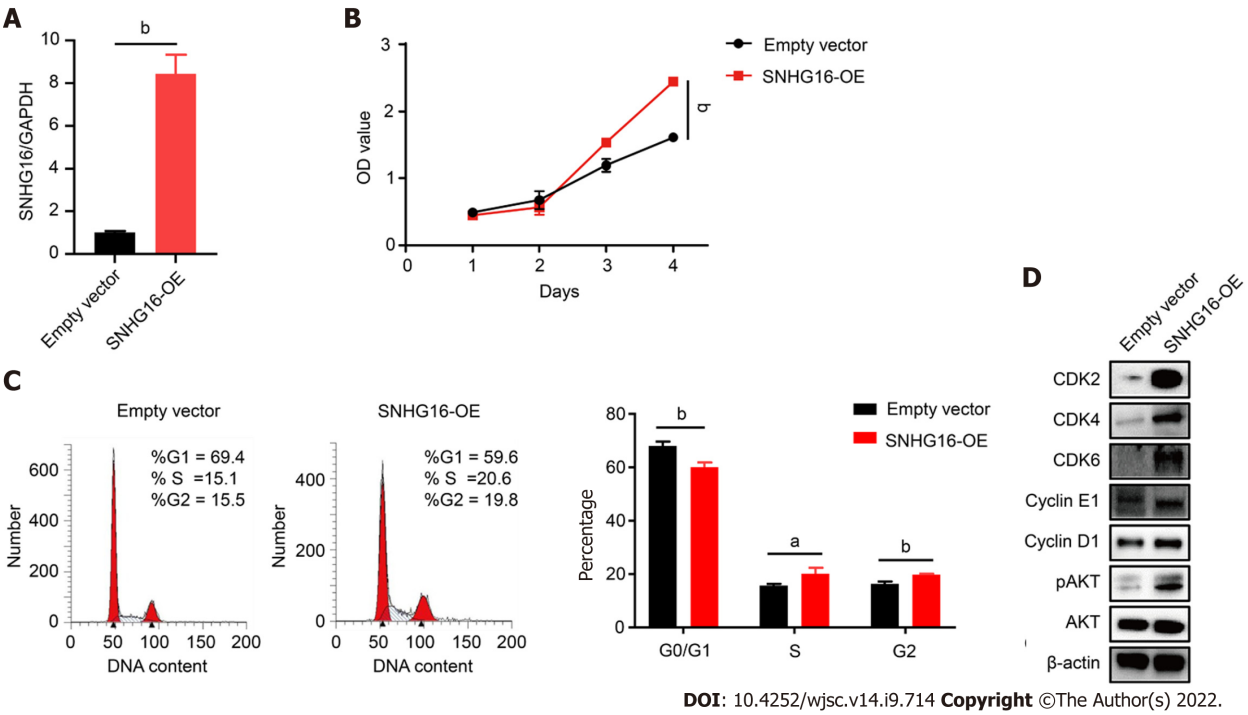
The results of the bioinformatic analysis were consistent with our experimental results. Combined with the individual analysis of upregulated genes, we found that hypoxia affected multiple cellular functions, mainly through upregulating the expression of certain genes. Moreover, hypoxia could mediate cell cycle progression and activate the PI3K/AKT pathway. Similarly, lncRNA–mRNA co-expression network analysis indicated that *SNHG16*, a hypoxia-responsive lncRNA, is associated with key genes in the cell cycle or PI3K/AKT pathway. Therefore, *SNHG16* was selected as a potential promoter of the hP-MSC proliferative rate under hypoxia.

SNHG16 is a member of the SNHGs and is well-documented for its oncogenic properties in numerous types of malignancies[29]. *SNHG16* is reported to be involved in multiple cell biological functions, including cell cycle progression, proliferation, and migration[30-32]. In our study, we found that hypoxic culture could induce the expression of *SNHG16* in hP-MSCs. We further verified that *SNHG16* could promote cell cycle progression and cell proliferation of hP-MSCs by using knockdown and overexpression models. Moreover, we demonstrated that overexpression of *SNHG16* could increase the phosphorylation of AKT with a simultaneous elevation in the expression levels of G1 to S phase transition related proteins, including CDK2, CDK4, CDK6, cyclin E1, and cyclin D1.

However, how *SNHG16* becomes integrated in the PI3K/AKT signaling pathway in the study remains unknown. There are some related articles on the mechanism by which *SNHG16* regulates the AKT pathway in other models. For example, *SNHG16* could activate the PI3K/AKT pathway through *SNHG16*/*miR-338-3p*/*PLK4* axis in cisplatin-resistant neuroblastoma cells[33]. Moreover, *SNHG16* was found to facilitate proliferative diabetes-related abnormalities in cell proliferation through regulating *miR-7-5p*/*IRS1* to activate PI3K/AKT pathway in HG-stimulated hRMECs[31]. It can be seen that *SNHG16* mainly acts as a competing endogenous RNA to participate in the regulation of the PI3K/AKT signaling pathway. Our follow-up studies will take this as a starting point to elucidate the detailed mechanism of *SNHG16* regulation of the PI3K/AKT pathway.

CONCLUSION

In this study, we have shown that hypoxia enhanced hP-MSCs proliferation ability and could



DOI: 10.4252/wjsc.v14.i9.714

Figure 6 *SNHG16* overexpression resulted in activation of the PI3K/AKT pathway and a significant enhancement in the proliferative rate of human placenta-derived mesenchymal stem cells. A: Quantitative reverse transcription polymerase chain reaction analysis of relative *SNHG16* expression after transfection of lentivirus overexpressing *SNHG16* (*SNHG16*-OE) and the corresponding empty vector in human placenta-derived mesenchymal stem cells; B: Cell proliferation after *SNHG16* overexpression was evaluated by cell counting kit-8 assay; C: Cell cycle distribution after *SNHG16* overexpression was evaluated by flow cytometry; D: The expression levels of CDK2, CDK4, CDK6, cyclin E1, cyclin D1, and phosphorylated AKT. Data are presented as the means ± SD obtained from three separate experiments. $^{b}P < 0.01$.

specifically alter the lncRNA and mRNA expression profile. Furthermore, we identified a hypoxia-responsive lncRNA, *SNHG16*, which may serve as a regulator of promoting hP-MSCs proliferation under hypoxia. Mechanically, *SNHG16* was shown to activate the PI3K/AKT signaling pathway and upregulate the expression of key cell cycle regulators to induce cell cycle transition.

ARTICLE HIGHLIGHTS

Research background

As the role of hypoxia on mesenchymal stem cells (MSCs) is an emerging topic of MSCs biology, increasing studies are devoted to researching the regulation mechanisms of hypoxia on the biological functions of MSCs. Long non-coding RNAs (lncRNAs) and messenger RNAs (mRNAs) are reported to possess a critical role in regulating MSC biological characteristics. Nonetheless, the specific expression and co-expressed profiles of lncRNAs and mRNAs in human placenta-derived MSCs (hP-MSCs) under hypoxia and underlying mechanism of lncRNAs on hP-MSCs biology are still unknown.

Research motivation

Although some studies have explored the effects of hypoxia on MSCs, the role of lncRNAs in them remains unclear.

Research objectives

In this study, we aimed to reveal the specific expression profiles of lncRNAs in hP-MSCs under hypoxia and initially explored the possible mechanism of lncRNAs on hP-MSCs biology.

Research methods

Here, we used a multigas incubator (92.5% N_2, 5%CO_2 and 2.5% O_2) to mimic a hypoxia condition and observed that hypoxic culture can significantly promote the proliferation potential of hP-MSCs. RNA sequencing technology was applied to identify the exact expression profiles of lncRNAs and mRNAs under hypoxia. After establishment of *SNHG16*-knockdown and *SNHG16*-overexpression hP-MSCs, the effect of *SNHG16* on proliferation capacity of hP-MSCs was analyzed *via* cell counting kit-8 and cell cycle analysis. Finally, the underlying mechanism was analyzed by western blot.

Research results

We identified 289 differentially expressed lncRNAs and 240 differentially expressed mRNAs between hypoxia group and normoxia group. Among them, the lncRNA *SNHG16* was upregulated under hypoxia, which was also validated by reverse transcription polymerase chain reaction. *SNHG16* was confirmed to affect hP-MSCs proliferation rates by studying the *SNHG16* knockdown model. *SNHG16* overexpression could significantly enhance proliferation capacity of hP-MSCs, activate PI3K/AKT pathway, and upregulate the expression of cell cycle-related proteins.

Research conclusions

Our results revealed the specific expression characteristics of lncRNAs and mRNAs in hypoxia-cultured hP-MSCs and identified that hypoxia-responsive lncRNA *SNHG16* can promote hP-MSC proliferation through the PI3K/AKT pathway.

Research perspectives

This study may contribute to understanding the role of noncoding RNAs in MSC biology.

FOOTNOTES

Author contributions: Feng XD contributed to the study design, experiments, data collection, and manuscript writing; Zhou JH, Chen JY, Feng B, Hu RT, and Wu J contributed to data collection and analysis; Pan QL, Yang JF and Yu J contributed to revising the manuscript; Cao HC contributed to design of the study, revision of the manuscript, and funding acquisition; All authors have read and approved the final manuscript.

Supported by Stem Cell and Translational Research from National Key Research and Development Program of China, No. 2020YFA0113003; and National Natural Science Foundation of China, No. 81971756.

Institutional review board statement: All protocols for the processing of human tissues and cells were approved by the Ethics Committee of The First Affiliated Hospital of Zhejiang University, No. 2020-1088.

Informed consent statement: Informed consent was waived.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The data presented in this study are available within the article text and figures.

Country/Territory of origin: China

ORCID number: Xu-Dong Feng 0000-0002-4103-7737; Jia-Hang Zhou 0000-0003-0623-517X; Qiao-Ling Pan 0000-0002-3771-8193; Jin-Feng Yang 0000-0001-6284-1700; Jiong Yu 0000-0002-1821-1178; Hong-Cui Cao 0000-0002-6604-6867.

S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Zhang YL

REFERENCES

1 **El Agha E**, Kramann R, Schneider RK, Li X, Seeger W, Humphreys BD, Bellusci S. Mesenchymal Stem Cells in Fibrotic Disease. *Cell Stem Cell* 2017; **21**: 166-177 [PMID: 28777943 DOI: 10.1016/j.stem.2017.07.011]
2 **Lightner AL**, Faubion WA. Mesenchymal Stem Cell Injections for the Treatment of Perianal Crohn's Disease: What We Have Accomplished and What We Still Need to Do. *J Crohns Colitis* 2017; **11**: 1267-1276 [PMID: 28387832 DOI: 10.1093/ecco-jcc/jjx046]
3 **Hu C**, Zhao L, Zhang L, Bao Q, Li L. Mesenchymal stem cell-based cell-free strategies: safe and effective treatments for liver injury. *Stem Cell Res Ther* 2020; **11**: 377 [PMID: 32883343 DOI: 10.1186/s13287-020-01895-1]
4 **Zhu R**, Yan T, Feng Y, Liu Y, Cao H, Peng G, Yang Y, Xu Z, Liu J, Hou W, Wang X, Li Z, Deng L, Wang S, Li J, Han Q, Li H, Shan G, Cao Y, An X, Yan J, Zhang Z, Qu X, Zhu J, Zhou S, Wang J, Zhang F, Gao J, Jin R, Xu D, Ma YQ, Huang T, Peng S, Zheng Z, Stambler I, Gilson E, Lim LW, Moskalev A, Cano A, Chakrabarti S, Ulfhake B, Su H, Xu H, Xu S, Wei F, Brown-Borg HM, Min KJ, Ellison-Hughes G, Caruso C, Jin K, Zhao RC. Mesenchymal stem cell treatment improves outcome of COVID-19 patients *via* multiple immunomodulatory mechanisms. *Cell Res* 2021; **31**: 1244-1262

[PMID: 34702946 DOI: 10.1038/s41422-021-00573-y]

5 **Shi L**, Wang L, Xu R, Zhang C, Xie Y, Liu K, Li T, Hu W, Zhen C, Wang FS. Mesenchymal stem cell therapy for severe COVID-19. *Signal Transduct Target Ther* 2021; **6**: 339 [PMID: 34497264 DOI: 10.1038/s41392-021-00754-6]

6 **Wang ZG**, He ZY, Liang S, Yang Q, Cheng P, Chen AM. Comprehensive proteomic analysis of exosomes derived from human bone marrow, adipose tissue, and umbilical cord mesenchymal stem cells. *Stem Cell Res Ther* 2020; **11**: 511 [PMID: 33246507 DOI: 10.1186/s13287-020-02032-8]

7 **Feng XD**, Zhu JQ, Zhou JH, Lin FY, Feng B, Shi XW, Pan QL, Yu J, Li LJ, Cao HC. Hypoxia-inducible factor-1α-mediated upregulation of CD99 promotes the proliferation of placental mesenchymal stem cells by regulating ERK1/2. *World J Stem Cells* 2021; **13**: 317-330 [PMID: 33959221 DOI: 10.4252/wjsc.v13.i4.317]

8 **Zhou J**, Feng X, Zhu J, Feng B, Yao Q, Pan Q, Yu J, Yang J, Li L, Cao H. Mesenchymal stem cell treatment restores liver macrophages homeostasis to alleviate mouse acute liver injury revealed by single-cell analysis. *Pharmacol Res* 2022; **179**: 106229 [PMID: 35470065 DOI: 10.1016/j.phrs.2022.106229]

9 **Meng F**, Xu R, Wang S, Xu Z, Zhang C, Li Y, Yang T, Shi L, Fu J, Jiang T, Huang L, Zhao P, Yuan X, Fan X, Zhang JY, Song J, Zhang D, Jiao Y, Liu L, Zhou C, Maeurer M, Zumla A, Shi M, Wang FS. Human umbilical cord-derived mesenchymal stem cell therapy in patients with COVID-19: a phase 1 clinical trial. *Signal Transduct Target Ther* 2020; **5**: 172 [PMID: 32855385 DOI: 10.1038/s41392-020-00286-5]

10 **Samal JRK**, Rangasami VK, Samanta S, Varghese OP, Oommen OP. Discrepancies on the Role of Oxygen Gradient and Culture Condition on Mesenchymal Stem Cell Fate. *Adv Healthc Mater* 2021; **10**: e2002058 [PMID: 33533187 DOI: 10.1002/adhm.202002058]

11 **Mohyeldin A**, Garzón-Muvdi T, Quiñones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. *Cell Stem Cell* 2010; **7**: 150-161 [PMID: 20682444 DOI: 10.1016/j.stem.2010.07.007]

12 **Li L**, Jaiswal PK, Makhoul G, Jurakhan R, Selvasandran K, Ridwan K, Cecere R. Hypoxia modulates cell migration and proliferation in placenta-derived mesenchymal stem cells. *J Thorac Cardiovasc Surg* 2017; **154**: 543-552.e3 [PMID: 28526501 DOI: 10.1016/j.jtcvs.2017.03.141]

13 **Hung SP**, Ho JH, Shih YR, Lo T, Lee OK. Hypoxia promotes proliferation and osteogenic differentiation potentials of human mesenchymal stem cells. *J Orthop Res* 2012; **30**: 260-266 [PMID: 21809383 DOI: 10.1002/jor.21517]

14 **Rosová I**, Dao M, Capoccia B, Link D, Nolta JA. Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. *Stem Cells* 2008; **26**: 2173-2182 [PMID: 18511601 DOI: 10.1634/stemcells.2007-1104]

15 **Wilusz JE**, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. *Genes Dev* 2009; **23**: 1494-1504 [PMID: 19571179 DOI: 10.1101/gad.1800909]

16 **Taniue K**, Akimitsu N. The Functions and Unique Features of LncRNAs in Cancer Development and Tumorigenesis. *Int J Mol Sci* 2021; **22** [PMID: 33435206 DOI: 10.3390/ijms22020632]

17 **Liu J**, Li P, Zhu J, Lin F, Zhou J, Feng B, Sheng X, Shi X, Pan Q, Yu J, Gao J, Li L, Cao H. Mesenchymal stem cell-mediated immunomodulation of recruited mononuclear phagocytes during acute lung injury: a high-dimensional analysis study. *Theranostics* 2021; **11**: 2232-2246 [PMID: 33500722 DOI: 10.7150/thno.52514]

18 **Phinney DG**, Kopen G, Isaacson RL, Prockop DJ. Plastic adherent stromal cells from the bone marrow of commonly used strains of inbred mice: variations in yield, growth, and differentiation. *J Cell Biochem* 1999; **72**: 570-585 [PMID: 10022616]

19 **Aust L**, Devlin B, Foster SJ, Halvorsen YD, Hicok K, du Laney T, Sen A, Willingmyre GD, Gimble JM. Yield of human adipose-derived adult stem cells from liposuction aspirates. *Cytotherapy* 2004; **6**: 7-14 [PMID: 14985162 DOI: 10.1080/14653240310004539]

20 **Elabd C**, Ichim TE, Miller K, Anneling A, Grinstein V, Vargas V, Silva FJ. Comparing atmospheric and hypoxic cultured mesenchymal stem cell transcriptome: implication for stem cell therapies targeting intervertebral discs. *J Transl Med* 2018; **16**: 222 [PMID: 30097061 DOI: 10.1186/s12967-018-1601-9]

21 **Mas-Bargues C**, Sanz-Ros J, Román-Domínguez A, Inglés M, Gimeno-Mallench L, El Alami M, Viña-Almunia J, Gambini J, Viña J, Borrás C. Relevance of Oxygen Concentration in Stem Cell Culture for Regenerative Medicine. *Int J Mol Sci* 2019; **20** [PMID: 30857245 DOI: 10.3390/ijms20051195]

22 **Barry FP**, Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. *Int J Biochem Cell Biol* 2004; **36**: 568-584 [PMID: 15010324 DOI: 10.1016/j.biocel.2003.11.001]

23 **Adesida AB**, Mulet-Sierra A, Jomha NM. Hypoxia mediated isolation and expansion enhances the chondrogenic capacity of bone marrow mesenchymal stromal cells. *Stem Cell Res Ther* 2012; **3**: 9 [PMID: 22385573 DOI: 10.1186/scrt100]

24 **Studer L**, Csete M, Lee SH, Kabbani N, Walikonis J, Wold B, McKay R. Enhanced proliferation, survival, and dopaminergic differentiation of CNS precursors in lowered oxygen. *J Neurosci* 2000; **20**: 7377-7383 [PMID: 11007896]

25 **Das R**, Jahr H, van Osch GJ, Farrell E. The role of hypoxia in bone marrow-derived mesenchymal stem cells: considerations for regenerative medicine approaches. *Tissue Eng Part B Rev* 2010; **16**: 159-168 [PMID: 19698058 DOI: 10.1089/ten.TEB.2009.0296]

26 **Meng SS**, Xu XP, Chang W, Lu ZH, Huang LL, Xu JY, Liu L, Qiu HB, Yang Y, Guo FM. LincRNA-p21 promotes mesenchymal stem cell migration capacity and survival through hypoxic preconditioning. *Stem Cell Res Ther* 2018; **9**: 280 [PMID: 30359325 DOI: 10.1186/s13287-018-1031-x]

27 **Gao H**, Dong H, Zheng J, Jiang X, Gong M, Hu L, He J, Wang Y. LINC01119 negatively regulates osteogenic differentiation of mesenchymal stem cells *via* the Wnt pathway by targeting FZD4. *Stem Cell Res Ther* 2022; **13**: 43 [PMID: 35093173 DOI: 10.1186/s13287-022-02726-1]

28 **Xu Y**, Xin R, Sun H, Long D, Li Z, Liao H, Xue T, Zhang Z, Kang Y, Mao G. Long Non-coding RNAs LOC100126784 and POM121L9P Derived From Bone Marrow Mesenchymal Stem Cells Enhance Osteogenic Differentiation *via* the miR-503-5p/SORBS1 Axis. *Front Cell Dev Biol* 2021; **9**: 723759 [PMID: 34746123 DOI: 10.3389/fcell.2021.723759]

29 **Zimta AA**, Tigu AB, Braicu C, Stefan C, Ionescu C, Berindan-Neagoe I. An Emerging Class of Long Non-coding RNA With Oncogenic Role Arises From the snoRNA Host Genes. *Front Oncol* 2020; **10**: 389 [PMID: 32318335 DOI: 10.3389/fonc.2020.00389]

30 **Jiang X**, Ru Q, Li P, Ge X, Shao K, Xi L, Xu B, Wang Q, Huang S. LncRNA SNHG16 induces proliferation and fibrogenesis *via* modulating miR-141-3p and CCND1 in diabetic nephropathy. *Gene Ther* 2020; **27**: 557-566 [PMID: 32504027 DOI: 10.1038/s41434-020-0160-x]

31 **Cai F**, Jiang H, Li Y, Li Q, Yang C. Upregulation of long non-coding RNA SNHG16 promotes diabetes-related RMEC dysfunction *via* activating NF-κB and PI3K/AKT pathways. *Mol Ther Nucleic Acids* 2021; **24**: 512-527 [PMID: 33898104 DOI: 10.1016/j.omtn.2021.01.035]

32 **Xia W**, Liu Y, Cheng T, Xu T, Dong M, Hu X. Extracellular Vesicles Carry lncRNA SNHG16 to Promote Metastasis of Breast Cancer Cells *via* the miR-892b/PPAPDC1A Axis. *Front Cell Dev Biol* 2021; **9**: 628573 [PMID: 34249903 DOI: 10.3389/fcell.2021.628573]

33 **Xu Z**, Sun Y, Wang D, Sun H, Liu X. SNHG16 promotes tumorigenesis and cisplatin resistance by regulating miR-338-3p/PLK4 pathway in neuroblastoma cells. *Cancer Cell Int* 2020; **20**: 236 [PMID: 32536824 DOI: 10.1186/s12935-020-01291-y]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2022 September 26; 14(9): 729-743

DOI: 10.4252/wjsc.v14.i9.729

ISSN 1948-0210 (online)

ORIGINAL ARTICLE

Basic Study

Intercellular mitochondrial transfer as a means of revitalizing injured glomerular endothelial cells

Li-Xia Tang, Bing Wei, Lu-Yao Jiang, You-You Ying, Ke Li, Tian-Xi Chen, Ruo-Fei Huang, Miao-Jun Shi, Hang Xu

Specialty type: Cell and tissue engineering

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Oliva J, United States; Prasetyo EP, Indonesia

Received: May 23, 2022
Peer-review started: May 23, 2022
First decision: July 6, 2022
Revised: July 18, 2022
Accepted: September 6, 2022
Article in press: September 6, 2022
Published online: September 26, 2022

Li-Xia Tang, You-You Ying, Ke Li, Ruo-Fei Huang, Department of Endocrinology, The First People's Hospital of Yongkang Affiliated to Hangzhou Medical College, Jinhua 321300, Zhejiang Province, China

Bing Wei, School of Medicine, Southeast University, Nanjing 210009, Jiangsu Province, China

Lu-Yao Jiang, Department of Medical Rehabilitation, The First People's Hospital of Yongkang Affiliated to Hangzhou Medical College, Jinhua 321300, Zhejiang Province, China

Tian-Xi Chen, Miao-Jun Shi, Department of Nephrology, The First People's Hospital of Yongkang Affiliated to Hangzhou Medical College, Jinhua 321300, Zhejiang Province, China

Hang Xu, Department of Hemodialysis/Nephrology, The First People's Hospital of Yongkang Affiliated to Hangzhou Medical College, Jinhua 321300, Zhejiang Province, China

Corresponding author: Hang Xu, BMed, Doctor, Occupational Physician, Department of Hemodialysis/Nephrology, The First People's Hospital of Yongkang Affiliated to Hangzhou Medical College, No. 599, Jinshan West Road, Dongcheng District, Jinhua 321300, Zhejiang Province, China. 13758989843@163.com

Abstract

BACKGROUND

Recent studies have demonstrated that mesenchymal stem cells (MSCs) can rescue injured target cells *via* mitochondrial transfer. However, it has not been fully understood how bone marrow-derived MSCs repair glomeruli in diabetic kidney disease (DKD).

AIM

To explore the mitochondrial transfer involved in the rescue of injured glomerular endothelial cells (GECs) by MSCs, both *in vitro* and *in vivo*.

METHODS

In vitro experiments were performed to investigate the effect of co-culture with MSCs on high glucose-induced GECs. The transfer of mitochondria was visualized using fluorescent microscopy. GECs were freshly sorted and ultimately tested for apoptosis, viability, mRNA expression by real-time reverse transcriptase-polymerase chain reaction, protein expression by western blot, and

mitochondrial function. Moreover, streptozotocin-induced DKD rats were infused with MSCs, and renal function and oxidative stress were detected with an automatic biochemical analyzer and related-detection kits after 2 wk. Kidney histology was analyzed by hematoxylin and eosin, periodic acid-Schiff, and immunohistochemical staining.

RESULTS

Fluorescence imaging confirmed that MSCs transferred mitochondria to injured GECs when co-cultured *in vitro*. We found that the apoptosis, proliferation, and mitochondrial function of injured GECs were improved following co-culture. Additionally, MSCs decreased pro-inflammatory cytokines [interleukin (IL)-6, IL-1β, and tumor necrosis factor-α] and pro-apoptotic factors (caspase 3 and Bax). Mitochondrial transfer also enhanced the expression of superoxide dismutase 2, B cell lymphoma-2, glutathione peroxidase (GPx) 3, and mitofusin 2 and inhibited reactive oxygen species (ROS) and dynamin-related protein 1 expression. Furthermore, MSCs significantly ameliorated functional parameters (blood urea nitrogen and serum creatinine) and decreased the production of malondialdehyde, advanced glycation end products, and ROS, whereas they increased the levels of GPx and superoxide dismutase *in vivo*. In addition, significant reductions in the glomerular basement membrane and renal interstitial fibrosis were observed following MSC treatment.

CONCLUSION

MSCs can rejuvenate damaged GECs *via* mitochondrial transfer. Additionally, the improvement of renal function and pathological changes in DKD by MSCs may be related to the mechanism of mitochondrial transfer.

Key Words: Mitochondria transfer; Mesenchymal stem cells; Glomerular endothelial cells; Diabetic kidney disease; Mitochondrial dysfunction; Oxidative stress

Core Tip: This study demonstrated that the MitoTracker Red CMXRos labeled mitochondria were transferred from mesenchymal stem cells (MSCs) to the high glucose-injured glomerular endothelial cells (GECs) *in vitro*. Additionally, GEC proliferation was enhanced, and GEC apoptosis was suppressed. Furthermore, *in vivo* experiments showed that MSCs ameliorated renal function damage and pathological progression of diabetic kidney disease (DKD). These data suggest that MSCs may rescue damaged GECs and improve the renal function and pathological changes of DKD partly through mitochondrial transfer.

Citation: Tang LX, Wei B, Jiang LY, Ying YY, Li K, Chen TX, Huang RF, Shi MJ, Xu H. Intercellular mitochondrial transfer as a means of revitalizing injured glomerular endothelial cells. *World J Stem Cells* 2022; 14(9): 729-743

URL: https://www.wjgnet.com/1948-0210/full/v14/i9/729.htm

DOI: https://dx.doi.org/10.4252/wjsc.v14.i9.729

INTRODUCTION

The prevalence of diabetic kidney disease (DKD), also known as diabetic nephropathy, is increasing worldwide. The global all-age mortality rate from chronic kidney disease (CKD) increased by 41.5% between 1990 and 2017[1]. Additionally, CKD caused the death of 1.2 million people in 2017 and there was an increase of 697.5 million cases of all-stage CKD[1]. Furthermore, a decomposition analysis showed that the burden of DKD accounted for about half the increase in CKD disability-adjusted life years[2]. Due to the high mortality rate, morbidity, and financial burden, DKD is an urgent public health issue.

Amongst the many known mechanisms of DKD pathophysiology, the mechanism of mitochondrial dysfunction appears to play an essential role in its development[3,4]. Mitochondria play vital roles in biological processes such as oxidative phosphorylation, cellular metabolism, and cell death[5]. Recent studies indicate that mitochondrial damage occurs in glomerular endothelial cells (GECs) and podocytes in DKD[6,7]. Moreover, hyperglycemia results in mitochondrial dysfunction[8], which produces an excessive amount of reactive oxygen species (ROS), especially in GECs[6].

Mesenchymal stem cells (MSCs) have the potential to treat diabetes-related complications. However, their therapeutic effects and mechanisms of action have not been determined as of yet. Notably, a

possible benefit of stem cells might be their ability to release mitochondria[9]. Transferring mitochondria from human bone marrow MSCs (BMSCs) to human umbilical cord vein endothelial cells (HUVECs) has been suggested to reduce apoptosis, stimulate proliferation, and restore transmembrane migration in injured HUVECs[10]. In streptozotocin (STZ)-induced diabetic animals, Konari reported the transfer of mitochondria from systemically administered BMSCs to renal proximal tubular epithelial cells (PTECs)[11]. Moreover, when BMSCs transferred their mitochondria to lung epithelial cells[12] and cardiomyocytes[13], they resulted in increased adenosine triphosphate (ATP) levels and apoptosis suppression. In addition, MSCs' mitochondria can be transferred to myocardial cells[14], alveolar epithelial cells[15], and astrocytes[16], which restore cellular oxidative respiratory function and reduce apoptosis. Therefore, a new concept for cell-cell signals involving intercellular mitochondrial transfer is now proposed[9]. Because the pathological changes in many tissues are related to the impairment of mitochondrial function, replacing dysfunctional mitochondria with healthy donor mitochondria has broad research applications[16]. Supplementing exogenous healthy mitochondria to replace damaged mitochondria can improve the bioenergetics of damaged cells, reverse excessive ROS production, and restore mitochondrial function[17]. However, experimental data on how stem cells influence injured GEC mitochondria is limited.

Previous studies have focused on glomerular hyper-filtration, oxidative stress, advanced glycation end products (AGEs), activation of intracellular signaling pathways, and epigenetic changes in the pathogenesis of DKD[2]. However, dysregulation of mitochondrial metabolism leads to the occurrence and progression of DKD[7,18]. The importance of MSCs in glomerular development is still highly debated, but one theory is that MSCs provide an environment conducive to glomerular development. Further investigation of the mechanisms of action of MSCs should be conducted, particularly those that involve the interaction between GECs and grafted MSCs. This study was designed to determine if MSCs could repair GECs with dysfunctional mitochondria by transferring their mitochondria. This research supports the idea that stem cell mitochondrial transfer can treat DKD or other diseases with mitochondrial dysfunction.

MATERIALS AND METHODS

Cell cultures and cell lines

BMSCs were isolated from Sprague-Dawley rats (4-6 wk old) using the adherence exclusion method following previously published protocols[19]. The isolated cells were resuspended in endothelial cell medium (ECM) (ScienCell, California, United States) containing 10% (v/v) fetal bovine serum (FBS) (ScienCell) and 1% (v/v) penicillin-streptomycin (P/S) (ScienCell), and then incubated at 37 °C with 5% circulating CO_2. BMSCs at passages 2-4 were used in the following experiments. GECs were purchased (ScienCell) and cultured in ECM containing 10% FBS, 1% P/S, and 1% (v/v) endothelial cell growth supplement (ScienCell). GECs at passages 2-3 were used in the following experiments.

BMSCs were identified by differentiation potential and fluorescence-activated cell sorting (FACS) to evaluate the cell surface markers. BMSCs could be differentiated into osteogenic, adipogenic, and chondrogenic phenotypes when incubated in an osteogenic-, adipogenic-, or chondrogenic-inducing medium (Cyagen, Suzhou, China) according to the manufacturer's instructions. Osteogenic, adipogenic, and chondrogenic differentiation capacity of BMSCs was observed using Alizarin red staining, Oil red O staining, and Alcian Blue staining, respectively, and photographed under the light microscope (Olympus, Tokyo, Japan). The results are shown in Supplementary Figures 1A-C. The BMSCs were incubated with PE-conjugated CD45 polyclonal antibody (BD Biosciences, United States) and fluorescein isothiocyanate (FITC)-conjugated CD44 polyclonal antibody (BD Biosciences). The BMSCs expressed the antigen CD44 but not CD45 (Supplementary Figure 1D).

Cell label and co-culture model

The mitochondria of GECs and BMSCs were labeled to detect mitochondrial transfer before co-cultivation. GEC cells (5×10^5 cells) were first incubated with 200 nmol/L MitoTracker Green (Beyotime, Shanghai, China) for 25 min at 37 °C with 5% CO_2, and then the nuclei were stained with Hoechst 33342 (Beyotime). BMSCs (5×10^5 cells) were incubated with 200 nmol/L MitoTracker Red CMXRos (Beyotime) for 30 min, and then co-cultured with GECs in a 1:1 ratio and incubated at 37 °C with 5% CO_2. GECs were pre-cultured in ECM complete medium supplemented with high D-glucose (30 mmol/L; Sigma, United Kingdom) for 24 h to induce stress. Cells were randomly divided into four groups: (1) Normal control (NC) group: ECM complete medium containing D-glucose (5.5 mmol/L); (2) NC + MSC group; (3) High glucose (HG) group: D-glucose (30 mmol/L); and (4) HG + MSC group. A fluorescence microscope (Olympus) was used to examine live cells after 48 h of co-culture. For further analysis, supernatants and cells were collected.

Flow cytometry and cell sorting

To examine the protective effects on the injured GECs, FACSAria III analysis (BD Biosciences) was used on at least 2×10^7 co-cultured cells after 48 h. GECs requiring sorting were pre-labeled with

CellTracker™ Violet (CTV) (Invitrogen, United States). Sorting and purification were performed based on CTV-positive labeled cells. Following the cell sorting, GECs were tested for apoptosis, viability, ROS measurement, protein expression by western blot, and mitochondrial function.

Measurement of cell apoptosis and viability

Annexin V-FITC/Propidium Iodide (PI) Apoptosis Detection Kit (Beyotime) was used to determine apoptosis after co-culturing for 48 h. The sorted GECs were resuspended in 195 μL Annexin-binding buffer according to the manufacturer's instructions. In brief, approximately 5 μL of Annexin V-FITC working solution and 10 μL of PI were added to a 1 × 10^5 cell suspension in darkness over 20 min. The fluorescence intensity was analyzed by BD FACSCelesta (BD Biosciences) within 1 h. Annexin V-FITC or PI staining results were calculated to indicate the early or late stages of apoptosis, respectively.

The cell viability was estimated using the Cell Counting Kit-8 (CCK-8) (Beyotime). GECs were plated in 10 μL of CCK-8 and incubated for 2 h in 96-well microplates filled with culture medium. A fluorescent microplate reader (FLx800™, BioTek, United Statets) was used to take readings at 450 nm, and a decrease in optical density was interpreted as a decrease in viability.

Assessment of ATP production

A luciferin-luciferase bioluminescence assay was used to assess ATP production. Briefly, the sorted GEC cells were collected, subjected to a single freeze-thaw cycle, and centrifuged. To measure ATP levels, supernatants were collected using an ATP determination kit (Invitrogen), following instructions from the kit manufacturer and the published protocol[20]. Standard curves normalized with protein concentrations (nmol/L ATP/μg protein) were used to calculate the ATP concentration.

Mitochondrial membrane potential

Mitochondrial membrane potential ($\Delta\Psi m$) was examined in live cells with the enhanced mitochondrial membrane potential assay kit (JC-1) (Beyotime). Briefly, 6 × 10^5 GEC cells were incubated with 0.5 mL JC-1 (1 ×) working solution in complete culture medium for 30 min at 37 °C and centrifuged at 600 g for 3 min. Then, the cells were resuspended with 1 mL JC-1 staining buffer. Carbonyl cyanide 3-chlorophenylhydrazone (10 μmol/L; Beyotime) treated cells were used as a positive control. The fluorescence intensity was analyzed by BD FACSCelesta (BD Biosciences) for quantitative analysis.

ROS and interleukin-6 measurement

Intracellular ROS and mitochondrial ROS were measured using flow cytometry, following cell staining with a DCF-DA probe (Beyotime) and MitoSOX™ Red fluorescent probe (Invitrogen), respectively. Freshly sorted GEC cells were seeded in a 48-well plate at a density of 1 × 10^4 cells per well and then cultured in a growth medium for 12 h to completely adhere to the wall surface. Cell pellets were collected after staining with DCF-DA (5 μmol/L) or MitoSOXTM Red (5 μmol/L) for 30 min at 37 °C, and fluorescence was detected with a flow cytometer. The level of interleukin (IL)-6 was measured according to the manufacturer's instructions for the enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen).

RNA extraction and real-time reverse transcriptase-polymerase chain reaction

Real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) was used to detect caspase 3, B cell lymphoma (*Bcl*)-2, *Bax*, tumor necrosis factor (*TNF*)-α, and *IL-1β* mRNA expression levels. Briefly, total RNA was isolated from cells or kidney tissues using Trizol reagent (Beyotime). With the Applied Biosystems™ 7500 RT-qPCR System (Thermo Fisher Scientific), RNA reverse transcription was performed with the SuperScript III (Invitrogen), followed by RT-qPCR with SYBR Green MasterMix. GAPDH served as an internal control. All samples were analyzed in triplicate. RT-qPCR results were analyzed by the $2^{-\Delta\Delta Ct}$ method and then converted to fold changes. All primer sequences were obtained from publications[21-24] and commercially synthesized (Servicebio, Wuhan, China), and the utilized sequences are shown in Supplementary Table 1.

Western blot analysis

After extracting the proteins with RIPA buffer and protease inhibitor, the total protein contents were measured using a BCA assay kit (Servicebio). By normalizing protein content, all samples had the same quality and volume for further analysis. A wet-transfer method was used to separate proteins by 10% or 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transfer them to polyvinylidene fluoride membranes. The membranes were blocked in pure methanol before use and then blocked in 1 × TBS containing 3% bovine serum albumin (Solarbio, Beijing, China). The membranes were then incubated overnight with primary antibodies: Anti-Bax (GB11690; Servicebio), anti-Bcl-2 (ab196495; Abcam), anti-caspase-3 (GB11767C; Servicebio), anti-superoxide dismutase 2 (SOD2) (GB111875; Servicebio), anti-glutathione peroxidase 3 (GPx-3) (ab256470; Abcam), anti-dynamin-related protein 1 (DRP1) (8570S; CST), anti-mitofusin 2 (MFN2) (ab124773; Abcam), and anti-β-Actin (GB15001; Servicebio) antibodies. After washing with TBS-T, they were incubated with appropriately diluted horseradish peroxidase (HRP) conjugated Goat Anti-Rabbit immunoglobulin (Ig)G (GB23303;

Servicebio) or HRP conjugated Goat Anti-Mouse IgG (GB23301; Servicebio) as the secondary antibody. Protein bands were visualized using the BeyoECL Moon chemiluminescence system (Beyotime).

Animal study

All animal methods were carried out following the National Institutes of Health Guidelines for the care and use of laboratory animals and were handled according to protocols approved by the Animal Experimental Ethical Committee of Southeast University (Nanjing, China). Eight-week-old male Sprague-Dawley rats were obtained from Southeast University Laboratory Animal Centre, and diabetes was induced by a single intraperitoneal injection of 60 mg/kg of STZ (Sigma-Aldrich, United States) dissolved in 10 mmol/L citrate buffer (pH 4.5), as our previously published study[25]. To verify the successful establishment of the diabetes model, fasting blood glucose (FBG) levels (≥ 16.7 mmol/L) were measured for three consecutive days after STZ administration for 3 d. The diabetic rats were provided with standard rat food for 4 wk. An excretion rate of > 30 mg of 24-h urinary albumin (U-Alb) was observed at week 4, suggesting successful DKD induction[26].

DKD rats were randomly divided into two groups (n = 5). Group 1 was treated with 2 × 10^6 MSCs (pre-labeled with 200 nmol/L MitoTracker Red CMXRos) dissolved in 100 μL Hank's Balanced Salt Solution (HBSS) (DKD + MSC group), and group 2 received HBSS (DKD group) by tail vein injection. Five non-diabetic rats served as a normal control group (NC group). Rats were sacrificed 2 wk after treatment for biochemical and histological analyses.

Evaluation of FBG, 24 h U-Alb, serum blood urea nitrogen, serum creatinine, and AGE levels

The levels of FBG were measured before and after each STZ injection. Samples obtained from the tail vein were tested for FBG using a blood glucose meter (LifeScan, CA, United States). Metabolic cages were used to collect 24-h urine from rats. The levels of 24 h U-Alb, blood urea nitrogen (BUN), and serum creatinine (Scr) were measured using an AU2700 automatic biochemical analyzer (Olympus). AGE level was measured according to the ELISA kit's instructions (Cusabio, China).

Assessment of SOD, malondialdehyde, and GPx in kidney tissue

A homogenizer was used to homogenize approximately 100 mg of kidney tissue in 5% phosphate-buffered saline. After centrifugation, the clear supernatant was collected. SOD values were measured with the xanthine oxidase activity assay kit (Sigma-Aldrich), the malondialdehyde (MDA) level was measured by the thiobarbituric acid method (MDA colorimetric assay kit; Elabscience, China), and a colorimetric assay kit (Elabscience) was used to measure the GPx concentration, following the manufacturer's protocol.

Histological analysis

Rat kidneys were dissected and fixed in 4% paraformaldehyde and embedded in paraffin, and 4 μm serial sections were then prepared for histological analysis under a light microscope or fluorescence microscope. TdT-mediated dUTP nick-end labeling (TUNEL) was done with an apoptosis detection kit (Servicebio) following the manufacturer's instructions. Hematoxylin and eosin (HE) staining and periodic acid-Schiff (PAS)[27] staining were carried out using standard protocols. A random sample of three glomeruli from each rat was analyzed using image analysis software (Image-Pro plus 6.0) to determine the percentage of PAS-positive areas, expressed as a mesangial index. Immunohistochemical (IHC) analyses were carried out using the rabbit anti-AGEs (1:300; bs-1158R; Bioss, Woburn, MA, United States) primary antibodies. HRP-conjugated goat anti-rabbit IgG (1:500; GB23303; Servicebio) was used to detect primary antibodies. The nuclei were subsequently stained with 3,3′-diaminobenzidine, and three fields of view from each rat were digitized. The integrated optical density from all fields was calculated using Image-Pro Plus 6.0.

Statistical analysis

Data are presented as the mean ± SD based on at least three independent experiments. Statistical analyses were conducted using IBM SPSS 26.0 (Chicago, IL, United States). Comparing multiple groups was done using one-way analysis of variance, followed by Bonferroni's *post hoc* test. The significance of the data was defined at $P < 0.05$.

RESULTS

Transfer of mitochondria from MSCs to GECs alleviates high glucose-induced GEC apoptosis and promotes GEC proliferation

As a visual demonstration of mitochondrial transfer, the mitochondria of the GECs and the BMSCs were labeled with MitoTracker Green and MitoTracker Red CMXRos, respectively, and the nuclei of GECs were labeled with Hoechst 33342 before co-cultivation. CMXRos-labeled mitochondria were rarely transferred from MSCs to NC-GECs. However, CMXRos-mitochondria of MSCs were markedly

transferred into HG-induced damaged GECs *in vitro* (Figure 1A).

To further confirm that mitochondrial transfer is involved in protecting GECs, RT-qPCR, CCK-8, western blot, and flow cytometry assays were performed. The mRNA and protein expression levels of caspase 3 and Bax in the HG group were dramatically higher than those in the control group (Figures 1B and C). Interestingly, treatment with MSCs significantly inhibited this upregulation (Figures 1B and C). The changes observed in the mRNA and protein expression of Bcl-2 were opposite to those observed for Bax and caspase 3 (Figures 1B and C). The ratio of Bcl-2/Bax increased in the HG + MSC group compared to that in the HG group, although it was not statistically significant. Flow cytometry with Annexin V-FITC/PI staining and CCK-8 assay was used to detect cell apoptosis and viability, respectively. From these assessments, we observed that HG treatment significantly reduced the cell viability of cultured GECs and increased their apoptosis (NC *vs* HG: 0.533 ± 0.053 *vs* 0.336 ± 0.043, $P < 0.001$). In contrast, MSC co-culture significantly reversed these outcomes (HG *vs* HG + MSC: 0.3358 ± 0.043 *vs* 0.439 ± 0.05, $P < 0.01$) (Figures 1D and E). These results suggest that mitochondrial transfer plays a notable role in the anti-apoptotic mechanisms of MSCs.

Mitochondrial transfer alleviates mitochondrial activity and oxidative stress in high glucose-induced GECs

To evaluate the mitochondrial activity of GECs, *in vitro* co-culture experiments were performed. CTV-positive GECs were sorted by flow cytometry. Compared with the HG-induced GECs group, the ATP production of MSC-treated GECs was significantly upregulated (5.59 ± 0.58 *vs* 6.91 ± 0.84, $P < 0.05$) (Figure 2A). Additionally, staining with the $\Delta\Psi m$ indicator, JC-1, revealed significant attenuation of GECs by flow cytometry at 48 h (HG *vs* HG + MSC: 69.21 ± 5.06 *vs* 80.44 ± 6.49, $P < 0.05$) (Figure 2B).

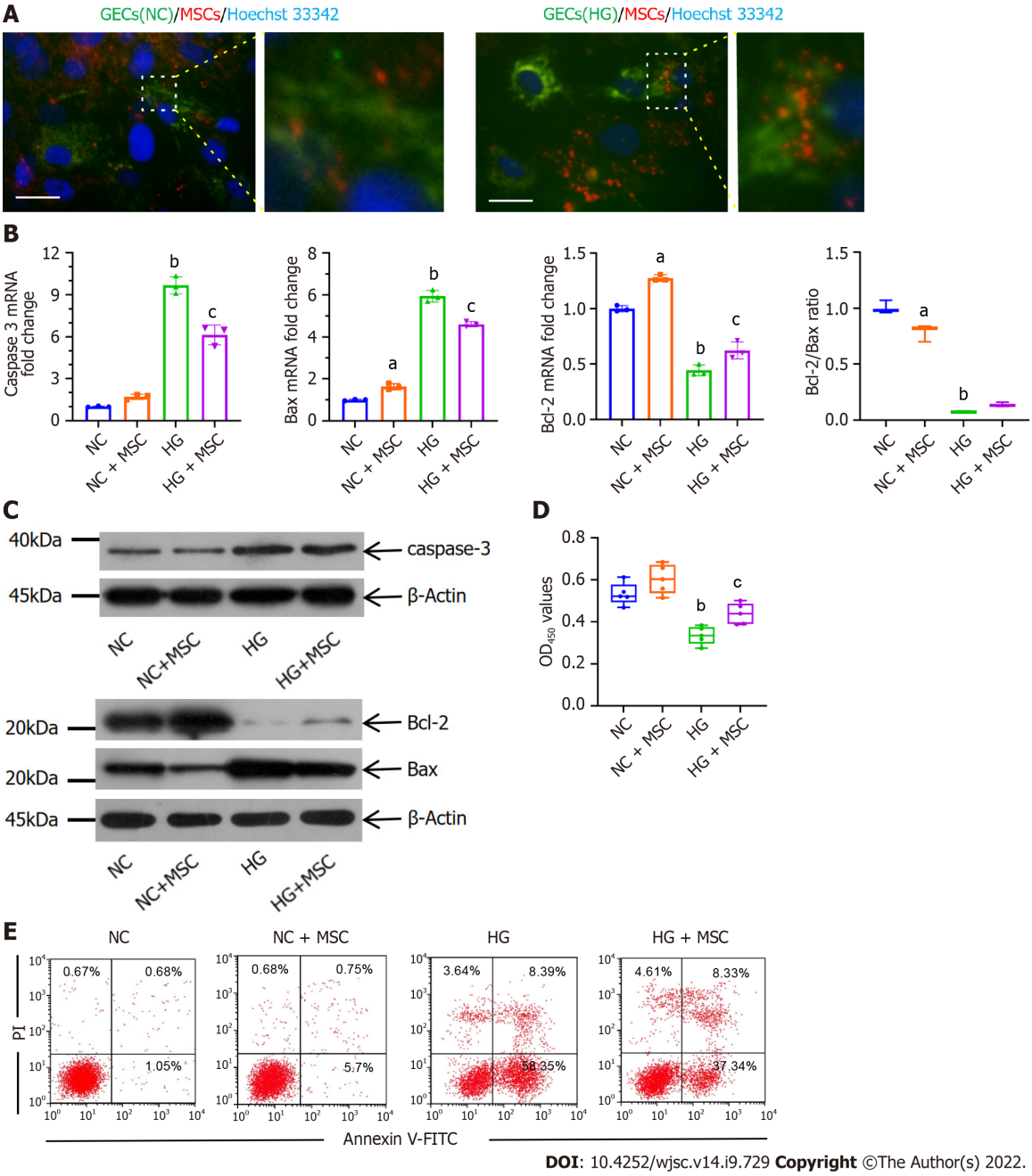
To determine the impact of mitochondrial transfer on mitochondrial dynamics, the protein levels of mitochondrial fission factor (DRP1) and fusion factor (MFN2) were analyzed. Representative western blots showed that treatment with MSCs decreased the level of DRP1, whereas MFN2 level increased (Figure 2C). In addition, intracellular and mitochondrial ROS production was visualized using DCF-DA and MitoSOX Red ROS indicators, respectively. Compared with NC-GECs, HG significantly increased DCF-DA and MitoSOX Red fluorescent intensity, suggesting high levels of ROS. In contrast, pretreatment with MSCs attenuated HG-induced upregulation in ROS levels (Figure 2D). Moreover, the expression levels of ROS-protective enzymes (SOD2 and GPx-3) increased after MSC treatment of HG-induced GECs (Figure 2E). We then analyzed the expression of inflammatory cytokines in GECs cultured with or without MSCs to characterize the mechanism of mitochondrial transfer on HG-GECs. The expression of the inflammatory cytokines TNF-α, IL-1β, and IL-6 decreased significantly in the HG-GECs cultured with MSCs for 48 h. In contrast, the addition of MSCs did not affect the expression of these inflammation cytokines in NC-GECs (Figures 2F and G). The above results demonstrate that direct co-culture with MSCs improved the capacity of GECs to resist oxidative stress.

MSC treatment improves renal function and relieves inflammation of DKD rats

An animal model of STZ-induced DKD was established to explore the therapeutic effect of MSCs on DKD. Following MSC treatment for 2 wk, the rats were sacrificed (5/5 in each group), and tissue specimens were collected for further analysis (Figure 3A). The FBG and 24 h U-Alb levels of the DKD and DKD + MSC groups were significantly higher than those of the NC group. There was no statistically significant increase in FBG or 24 h U-Alb in the MSC group relative to the DKD group, although the levels showed a trend towards an increase (Figure 3B). Significantly higher serum BUN and Scr were found in the DKD group than in the control group, but MSC treatment significantly reduced these changes (Figure 3C). RT-qPCR was applied to evaluate the expression of apoptosis-related genes. Compared with the NC group, caspase 3 and Bax expression in DKD rats was significantly increased, suggesting increased pro-apoptotic mechanisms (Figure 3D). In contrast, MSC injection reduced the expression of the pro-apoptotic markers caspase 3 and Bax. However, changes in the expression of Bcl-2 were increased (Figure 3D). The ratio of Bcl-2/Bax increased in the DKD + MSC group compared to the DKD group, although the difference was not statistically significant. The levels of MDA (a marker of lipid peroxidation/oxidative stress) and AGEs (contribute to oxidative stress) were lower in the NC group than in the other groups (Figure 3E). MSCs significantly decreased AGEs and MDA in DKD rats (Figure 3E). Notably, the levels of GPx (a marker of oxidative stress) in each group were inversed (DKD *vs* DKD + MSC: 2.08 ± 0.29 *vs* 2.67 ± 0.2, $P < 0.05$) (Figure 3E). We then used DCF probes and the xanthine oxidase activity assay kit to evaluate the ROS generation and scavenging ability. ROS production decreased in the DKD group while SOD level increased after MSC administration (Figure 3F). Overall, these results suggest that MSCs can ameliorate the abnormal renal function of DKD rats.

MSC treatment ameliorates renal pathological changes

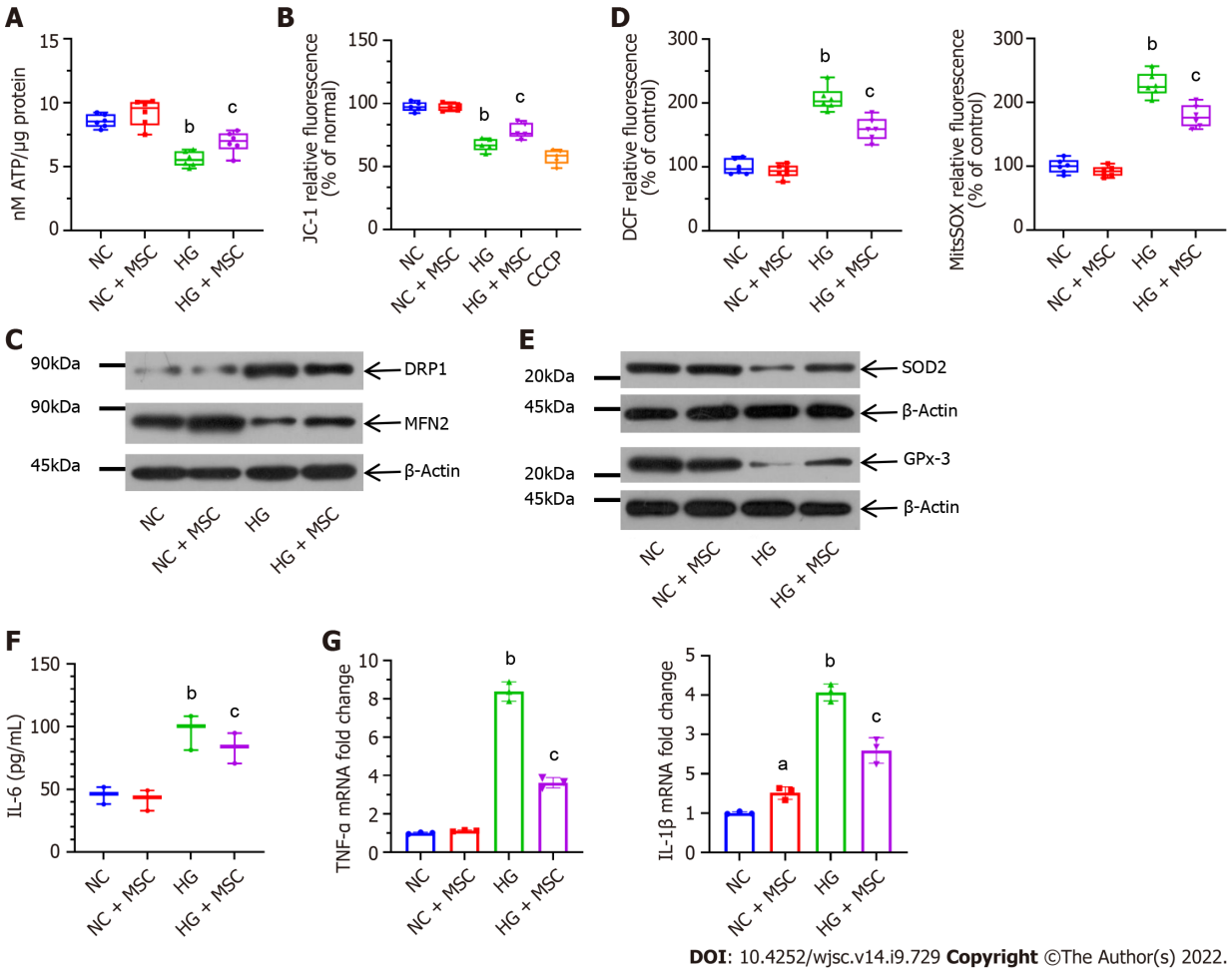
As shown in Figure 4, TUNEL, HE, PAS, and IHC staining (5/5 in each group) were performed on kidney tissue sections from selected experimental groups. The TUNEL method was used to investigate the apoptotic cells in renal tissue. Figure 4A shows that the number of TUNEL-positive apoptotic cells in kidney tissue of DKD rats was increased, while their positive expression was decreased after injection of



DOI: 10.4252/wjsc.v14.i9.729

Figure 1 Anti-apoptotic effects of mesenchymal stem cells on high glucose-induced glomerular endothelial cells *in vitro*. A: Immunofluorescence images of MitoTracker green (green) labeled normal control-glomerular endothelial cells (NC-GECs) and high glucose-induced GECs (HG-GECs) cultured with MitoTracker Red CMXRos (red) labeled mesenchymal stem cells (MSCs). GEC nuclei were counterstained with Hoechst 33342 (blue). A few spontaneous mitochondria transferred from MSCs to NC-GECs. Interestingly, a robust transfer of numerous mitochondria from MSCs to HG-GECs was observed. Scale bar: 200 nm; B: Caspase 3, *Bax*, and B-cell lymphoma 2 mRNA expression detected by real-time reverse transcriptase-polymerase chain reaction; C: Caspase 3, Bax, and B-cell lymphoma 2 protein expression detected by western blot; D: GEC viability assays performed using the Cell Counting Kit-8; E: Cellular apoptosis analysis in GEC cells treated with MSCs using Annexin V-FITC/PI staining. Data are presented as the mean ± SD. $^{a}P < 0.05$ *vs* normal control group, $^{b}P < 0.01$ *vs* normal control group, $^{c}P < 0.05$ *vs* high glucose-induced group. GECs: Glomerular endothelial cells; MSCs: Mesenchymal stem cells; NC-GECs: Normal control glomerular endothelial cells; HG-GECs: High glucose-induced GECs; Bcl-2: B-cell lymphoma 2.

MSCs. In the DKD group, HE staining revealed an inflammatory cell infiltration in the renal tissue (Figure 4B). Additionally, PAS staining showed severe glomerular and tubular changes in the DKD group. Atrophied glomeruli, ectopic mesangial extracellular matrix, high glycogen levels, kidney interstitial fibrosis, and basement membrane thickening were also observed (Figure 4C). The DKD group showed a degenerative phenotype indicative of glomerular endothelial degeneration by HE and PAS staining, while MSC therapy alleviated these pathological changes (Figures 4B and C). Compared to the NC group, the DKD and DKD + MSC groups showed significant increases in the mesangial index (58.69 ± 11.7 *vs* 195.13 ± 32.55, $P < 0.001$; 58.69 ± 11.7 *vs* 160.67 ± 29.12, $P < 0.001$; respectively) (Figure 4D). The mesangial index in the DKD + MSC group was markedly lower than that of the DKD



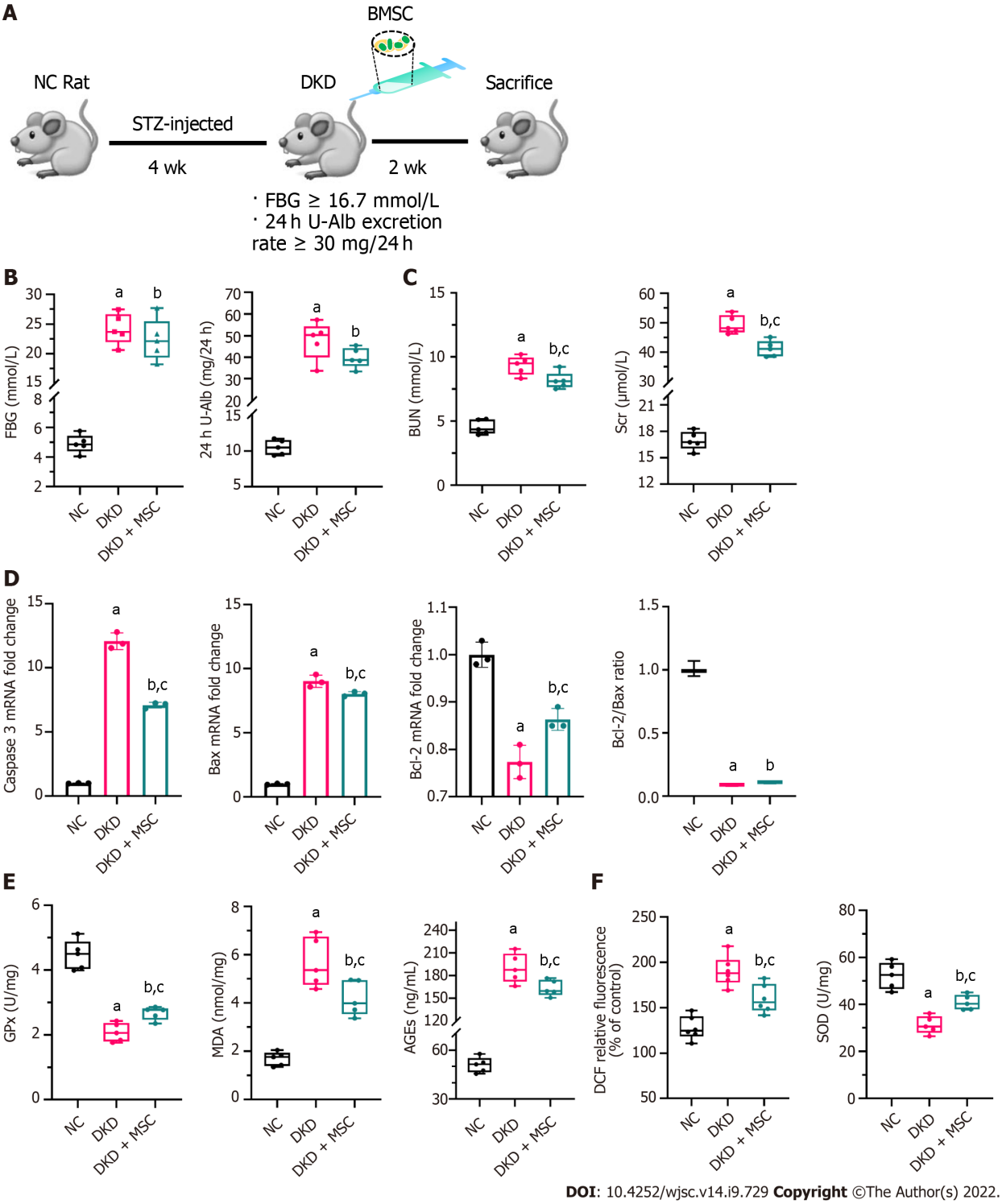
DOI: 10.4252/wjsc.v14.i9.729

Figure 2 Mesenchymal stem cells alleviate mitochondrial activity and high glucose-induced oxidative stress in cultured glomerular endothelial cells. A: Mesenchymal stem cells (MSCs) increased adenosine triphosphate production in high glucose (HG)-induced glomerular endothelial cells (GECs); B: MSCs also attenuated mitochondrial membrane potential ($\Delta\Psi m$) in GECs. Carbonyl cyanide 3-chlorophenylhydrazone as a mitochondrial oxidative phosphorylation uncoupler, was used as the positive control group; C: Effect on mitochondrial fission marker and the fusion marker levels; D: Pretreatment with MSCs attenuated HG-induced upregulation in DCF-DA [intracellular reactive oxygen species (ROS)] and MitoSOX Red (mitochondrial ROS) fluorescent intensity; E: Representative western blot expression levels of ROS-protective enzymes (superoxide dismutase 2 and glutathione peroxidase 3); F: Concentration of interleukin (IL)-6 in the supernatant by enzyme-linked immunosorbent assay; G: Mitochondrial transfer ameliorated the mRNA expression levels of inflammatory markers (tumor necrosis factor-α and IL-1β), as determined using real-time reverse transcriptase-polymerase chain reaction. Data are presented as the mean ± SD. $^{a}P < 0.05$ *vs* normal control group, $^{b}P < 0.01$ *vs* normal control group, $^{c}P < 0.05$ *vs* high glucose group. MSCs: Mesenchymal stem cells; DRP1: Dynamin-related protein 1; MFN2: Mitofusin 2; ROS: Reactive oxygen species; SOD2: Superoxide dismutase 2; GPx-3: Glutathione peroxidase 3; IL-6: Interleukin-6; CCCP: Carbonyl cyanide 3-chlorophenylhydrazone; NC: Normal control; HG: High glucose; TNF-α: Tumor necrosis factor-α; ATP: Adenosine triphosphate.

group (160.67 ± 29.12 *vs* 195.13 ± 32.55, $P < 0.01$). Figures 4E and F illustrates the kidney expression of AGEs as detected by IHC. A deficient level of staining signals was observed around the renal corpuscle wall and the tubular basement membrane in the NC group. Interestingly, the DKD group showed higher expression of AGEs than the NC group (64.53 ± 15.86 *vs* 8.58 ± 3.83, $P < 0.001$) or DKD + MSC group (64.53 ± 15.86 *vs* 52.62 ± 10.33, $P < 0.05$). Notably, the DKD + MSC group showed significantly decreased staining signals around the capsule and on the tubular basement membrane.

DISCUSSION

Recently, the administration of BMSCs has been shown to accelerate kidney reconstitution[28,29]. However, the underlying mechanism of action of MSCs that promote DKD kidney reconstitution is not yet fully understood. Apoptosis of GECs induced by mitochondrial dysfunction is suggested to play a role in the development of DKD. Therefore, one potential mechanism in BMSC-mediated kidney reconstitution is *via* BMSC and GEC cell-cell communication, resulting in the rescue of the injured GECs and promoting kidney reconstitution. MSCs can repair injuries in various ways, including secreting paracrine factors, transferring proteins and RNA, and transferring organelles such as mitochondria[30]. In this study, we demonstrated a novel mechanism of MSCs that they can transfer functional

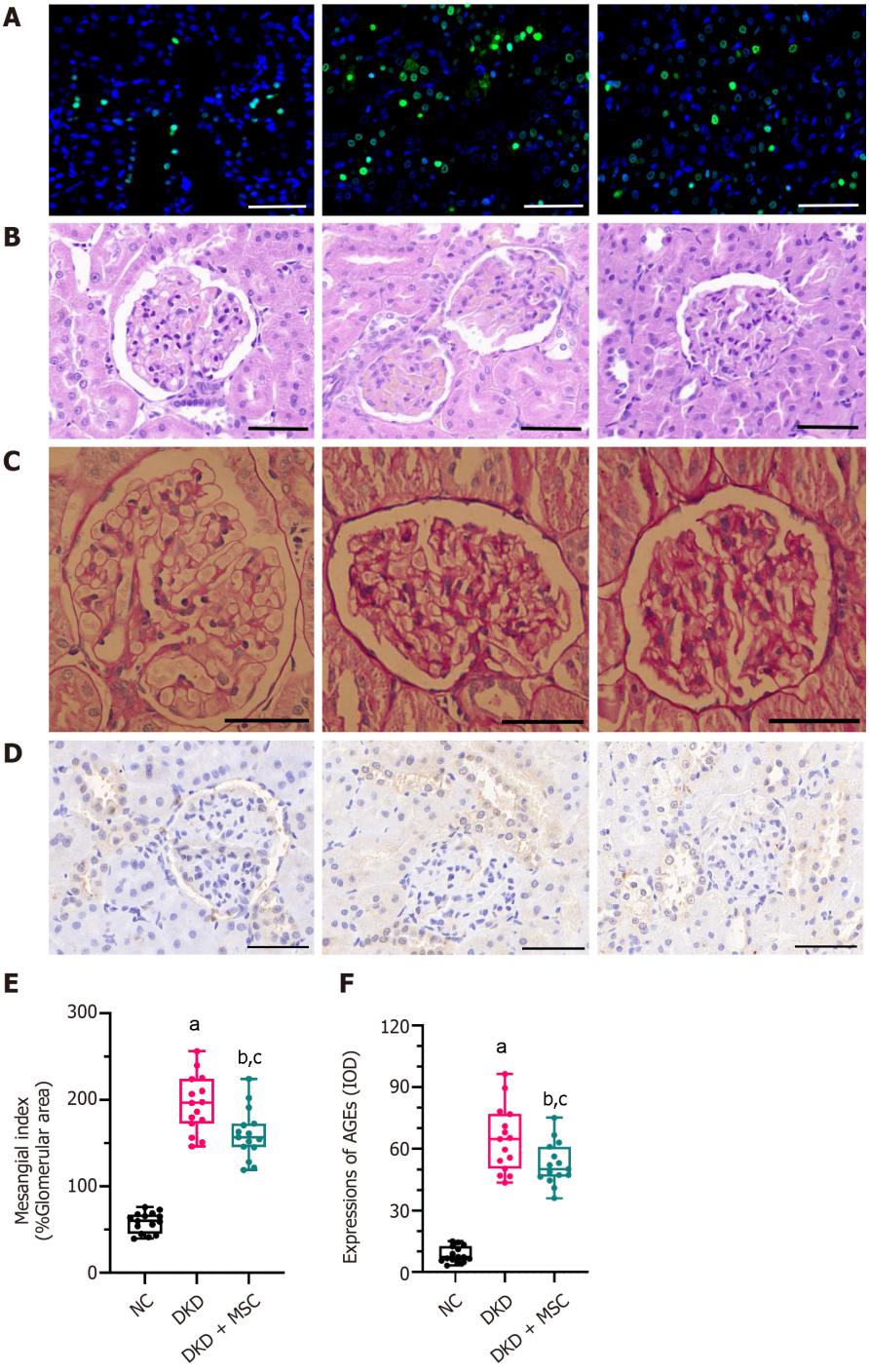


DOI: 10.4252/wjsc.v14.i9.729 **Copyright** ©The Author(s) 2022.

Figure 3 Effects of mesenchymal stem cell treatment on biochemical indexes. A: Flowchart of rat treatment from day 0 to week 6; B: Fasting blood glucose and 24-h urinary albumin concentration; C: Concentrations of blood urea nitrogen and serum creatinine in rat serum; D: Relative mRNA expression of caspase 3, *Bax*, and B-cell lymphoma 2 (Bcl-2), and Bcl-2/Bax ratio in rat kidney tissues; E: Concentrations of glutathione peroxidase, malondialdehyde, and advanced glycation end products in rat kidney tissues; F: Levels of reactive oxygen species and superoxide dismutase in kidney tissues. Data are presented as the mean ± SD. $^{a}P < 0.001$ *vs* normal control group, $^{b}P < 0.01$ *vs* normal control group, $^{c}P < 0.05$ *vs* diabetic kidney disease group. NC: Normal control; FBG: Fasting blood glucose; 24 h U-Alb: 24-h urinary albumin; BUN: Blood urea nitrogen; Scr: Serum creatinine; Bcl-2: B-cell lymphoma 2; Bax: BCL2-Associated X; GPx: Glutathione peroxidase; AGEs: Advanced glycation end products; MDA: Malondialdehyde; SOD: Superoxide dismutase; NC: Normal control; DKD: Diabetic kidney disease; MSCs: Mesenchymal stem cells.

mitochondria into GECs *in vitro*. Therefore, the therapeutic effects of BMSC on DKD rats may be related to the mechanism of mitochondrial transfer.

From our investigations, fluorescent imaging revealed that CMXRos-labeled mitochondria were transferred extensively from MSCs to HG-induced stressed GECs. As previously reported, the



DOI: 10.4252/wjsc.v14.i9.729

Figure 4 TdT-mediated dUTP nick-end labeling and histopathology analysis of kidney tissues from all rats. A: TdT-mediated dUTP nick-end labeling staining. Nuclei: Blue; apoptosis: Green; B: Hematoxylin-eosin staining; C: Periodic acid-Schiff (PAS) staining; D: Mesangial index of PAS-positive areas in the glomerulus; E: Immunohistochemical staining of advanced glycation end products (AGEs); F: Integrated optical density of AGEs in kidney tissues. All scale bar: 50 μm. Data are presented as the mean ± SD. $^{a}P < 0.001$ *vs* normal control group, $^{b}P < 0.001$ *vs* normal control group, $^{c}P < 0.05$ *vs* diabetic kidney disease group. AGEs: Advanced glycation end products; NC: Normal control; DKD: Diabetic kidney disease; MSCs: Mesenchymal stem cells.

mitochondria of BMSCs could be transferred to renal PTECs[11] and HUVEC cells[10]. Although recent studies have shown that mitochondrial transfer was bidirectional[31-33], our study did not find that the mitochondria of GECs were transferred to MSCs, which may be due to the difference in the recipient cell species. This finding is consistent with previous investigations[16,34-37] that demonstrated mitochondrial transfer from MSCs to injured target cells.

Mitochondria are energy factories that control cellular survival, stress, and apoptosis[38]. There has been evidence that MSCs can save damaged cells by transferring mitochondria, thus preventing tissue damage and regenerating metabolism[5,10,12]. Co-culturing with MSCs improved ATP production and $\Delta\Psi m$ of injured GECs. In addition, our results demonstrated that mitochondrial transferred from MSCs

to GECs could reduce apoptosis and promote proliferation in HG-stressed GECs. The finding that mitochondrial transfer reversed target cell proliferation and apoptosis was supported by a previous study conducted by Feng *et al*[10], which showed that MSCs promoted HUVEC proliferation and reduced HUVEC apoptosis through mitochondria transfer from MSCs to injured HUVECs. Additionally, hyperglycemia causes excessive oxidative stress, which contributes significantly to the pathogenesis of diabetic complications[3]. Apoptosis, ROS production, and defective mitophagy play crucial roles in DKD progression[3]. An elevated level of ROS is a biomarker of mitochondrial dysfunction in diabetic kidneys[3]. Fortunately, mitochondrial and intracellular ROS generation was inhibited with MSC supplementation, as well as the variation trend of SOD2 and GPx-3 levels. Furthermore, mitochondrial function was partially improved by MSC-mediated protection in *in vitro* investigations.

Recent studies indicate that mitochondrial dynamics (fusion and fission) play an essential role in mitochondrial distribution, maturation, and quality control[39]. In DKD rats, the fusion and fission of mitochondria are enhanced[4,40], which is similar to our finding that the expression of DRP1 decreased and that of MFN2 increased. Mitochondria play a role in cellular stress-induced apoptosis through various molecular mechanisms. Essentially, mitochondrial toxicity triggers the release of pro-apoptotic factors, which activate latent forms of caspases, resulting in cell death[3]. Importantly, hyperglycemia causes oxidative stress, which initiates caspase activation, leading to the release of TNF-α and activation of the mitochondria-mediated apoptotic pathway[41]. Furthermore, pro-apoptotic factors (caspase 3 and Bax) and inflammation-related factors (IL-6, TNF-α, and IL-1β) were down-regulated. In contrast, anti-apoptotic factors (Bcl-2) were upregulated after HG-injured GECs were co-cultured with MSCs. Additionally, the ratio of Bcl-2/Bax increased in the HG + MSC group compared to the HG group. However, these differences were minor and did not reach statistical significance. Possible reasons for this might include the following: (1) A too large control (NC and NC + MSC) group could lead to non-statistically significant differences in the HG and HG + MSC groups; and (2) This study had a small sample size, which may result in lower statistical power to detect differences between groups. These reasons may also be applicable to our *in vivo* experimentation and observations. In addition to their importance in intercellular mitochondrial transfer, these factors have also been associated with HG-related damage.

After MSC injection in STZ-induced DKD rats, there was no significant difference in the FBG level compared with the DKD group. This result is in agreement with previous reports[42,43], which may be related to the late treatment of MSCs and the missed opportunity to heal the acute pancreatic injury. Meanwhile, the level of 24 h U-Alb was not significantly different in the DKD + MSC group compared with the DKD group. The reason may be due to the small sample size or the short 2-wk duration of treatment. However, we did identify that the FBG and 24 h U-Alb levels in the DKD + MSC group were lower than those in the DKD group, although there was no statistical significance. Interestingly, a noteworthy finding was that MSCs effectively repaired renal dysfunction (BUN and Scr). Meanwhile, we observed a significant increase in GPx and SOD, indicating that MSCs can protect the kidneys from DKD.

Increased AGEs in DKD is another critical contributing factor resulting in mitochondrial dysfunction and apoptosis of GECs[44]. The main pathological change of DKD is glomerular lesions. A long-term and persistent high-glucose environment can activate protein kinase C and the renin-angiotensin system, induce accumulation of ROS and AGEs that damage endothelial cells, and generate proteinuria and glomerulosclerosis[45], which eventually aggravate renal function damage and progression of DKD. Furthermore, diabetics with glomerular damage may experience altered blood flow and oxygen delivery to other segments of the kidney[3]. However, treatment with MSCs resulted in renal histological changes manifested by reductions in glomerular volume, inflammatory cell infiltration, glomerular basement membrane, and renal interstitial fibrosis, consistent with previously reported results[42]. Therefore, MSCs can improve renal function and pathological changes in DKD to a certain extent.

Potential limitations of our study should be noted. First, further studies are warranted to explore and elucidate the mechanism of mitochondrial transfer from MSCs to injured GECs. For example, Liu *et al*[5] and Han *et al*[13] reported that MSCs transfer mitochondria to injured target cells *via* tunneling nanotubes, and this might be of interest to elucidate the exact mechanism of mitochondrial transfer. In addition, BMSCs can also transfer their mitochondria to target cells *via* gap junction channels containing the connexin 43 protein[12], extracellular vesicles[46], or endocytosis[47]. Second, since the laboratory conditions were unable to freeze renal tissue when they were obtained, the distribution of MSC mitochondria labeled with fluorescence in the tissue was not observed. Future studies are recommended to observe the map of MSC-derived mitochondria in DKD kidney tissue. Third, whether mitochondrial transfer from MSCs to GECs directly affects the function of GEC needs further verification by blocking the mitochondrial transfer. Fourth, TUNEL detects the apoptosis of cells in the whole kidney tissue, but it could not distinguish the apoptosis of GECs. Therefore, multiple fluorescent markers may be used for added analyses. Fifth, we did not assess the percentage of GECs with transferred MSC mitochondria. Our current objective focused on how to promote mitochondrial transfer from BMSC to GECs. Sixth, a study has shown that with intravenous injection of MSCs, these cells do not reach the damage site but release exosomes that can[48]. The article also reported that neither infusion of MSCs induced

significant fibrotic responses in organs (lungs, kidney, liver, and spleen), which might cause safety concerns. In our study, we did not assess the following issues: (1) The dynamic changes in the biodistribution of the BMSCs and the ratio/number of BMSCs after the injection; (2) The safety of the BMSC injection; and (3) The level of liver injury (aspartate aminotransferase, alanine aminotransferase, and others). However, we are optimistic about being able to solve these problems in our ongoing research.

CONCLUSION

Our study provides insights into the mechanisms underlying MSCs' ability to rescue injured GECs by a new cell-to-cell communication method of mitochondria transfer. Notably, mitochondria transfer alleviates mitochondrial damage and abates cellular apoptosis of GECs. Furthermore, the therapeutic effects of BMSC on DKD rats may be related to this mechanism of mitochondrial transfer.

ARTICLE HIGHLIGHTS

Research background

Mesenchymal stem cells (MSCs) can rescue injured target cells *via* mitochondrial transfer. However, little is known about how bone marrow-derived MSCs repair glomeruli in diabetic kidney disease (DKD).

Research motivation

Mitochondria play vital roles in biological processes such as oxidative phosphorylation, cellular metabolism, and cell death. Recent studies indicate that mitochondrial damage occurs in glomerular endothelial cells (GECs) in DKD and MSCs could transfer their mitochondria to target cells. However, the mechanism of how mitochondrial transfer contributes to the high glucose-injured GECs is not well-understood.

Research objectives

To investigate the mechanisms of mitochondrial transfer between MSC and high glucose-injured GECs or streptozotocin (STZ)-induced DKD rats.

Research methods

The mitochondria of GECs and MSCs were labeled before co-cultivation. A fluorescence microscope was used to examine the mitochondrial transfer, then cell proliferation and apoptosis were detected by western blot, real-time reverse transcriptase-polymerase chain reaction, Cell Counting Kit-8, and Annexin V-FITC/PI assays. The mitochondria function [adenosine triphosphate (ATP), reactive oxygen species (ROS), and mitochondrial membrane potential] of GECs was assessed with related-detection kits. A DKD rat model was obtained by STZ administration. Renal function and oxidative stress were detected with an automatic biochemical analyzer and related-detection kits. In addition, histological changes were evaluated by hematoxylin and eosin, periodic acid-Schiff, and immunohistochemical staining.

Research results

Our results demonstrated that the MitoTracker Red CMXRos labeled mitochondria were transferred from MSCs to the high glucose-injured GECs, ATP levels were increased, and the membrane potential of mitochondria was stabilized. Additionally, the transfer of mitochondria decreased pro-inflammatory cytokines [interleukin (IL)-6, IL-1β, and tumor necrosis factor-α] and pro-apoptotic factors (caspase 3 and Bax). Transfer of healthy MSC-derived mitochondria enhanced the expression of superoxide dismutase 2, B-cell lymphoma 2, glutathione peroxidase 3, and Mitofusin 2 and inhibited ROS (mitochondrial and intracellular) and dynamin-related protein 1 expression. Notably, a transfer of healthy mitochondria from MSCs suppressed GEC apoptosis and enhanced their proliferation. Furthermore, STZ-induced DKD animal experiments showed that MSC ameliorated renal function damage and pathological progression of DKD.

Research conclusions

Our data demonstrated the existing of mitochondrial transfer *in vitro*, which plays a pivotal role in the rescue of GECs. Moreover, MSCs repair the renal function damage and pathological progress of DKD rats perhaps *via* mechanism of mitochondrial transfer.

Research perspectives

This study revealed the role and mechanism of mitochondrial transfer in the rescue of injured GECs,

which can provide a scientific basis for the potential therapeutic effects of MSCs on DKD.

ACKNOWLEDGEMENTS

We express our sincere gratitude to our institution for its support and funding. Furthermore, all authors are grateful for the School of Medicine of Southeast University and its experimental platform.

FOOTNOTES

Author contributions: Tang LX, Wei B, Li K, and Xu H designed the study; Tang LX and Wei B wrote the manuscript; Tang LX, Wei B, Jiang LY, Ying YY, Chen TX, Huang RF, and Shi MJ performed the experiments; Tang LX, Wei B, Li K, and Xu H analysed the data; and all authors have read and approved the final manuscript.

Supported by the Science and Technology Foundation of Jinhua, No. 2021-4-190.

Institutional animal care and use committee statement: The animal experiments were carried out following the National Institutes of Health Guidelines for the care and use of laboratory animals and according to protocols approved by the Animal Experimental Ethical Committee of Southeast University (Nanjing, China). The animals were acclimatized to laboratory conditions (22-25 °C, 12 h/12 h light/dark, 50% humidity, *ad libitum* access to food and water). All animals were euthanized by 2%-3% halothane and then carbon dioxide inhalation for kidney tissue collection.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The datasets supporting the conclusions of this article are included within the article.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country/Territory of origin: China

ORCID number: Li-Xia Tang 0000-0002-5429-9146; Bing Wei 0000-0002-3398-8364; Lu-Yao Jiang 0000-0002-7745-2676; You-You Ying 0000-0001-5502-9087; Ke Li 0000-0002-4762-7711; Tian-Xi Chen 0000-0003-2112-3600; Ruo-Fei Huang 0000-0002-3032-7886; Miao-Jun Shi 0000-0001-9772-9268; Hang Xu 0000-0001-6304-5693.

S-Editor: Wang JJ
L-Editor: Wang TQ
P-Editor: Guo X

REFERENCES

1 **GBD Chronic Kidney Disease Collaboration**. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2020; **395**: 709-733 [PMID: 32061315 DOI: 10.1016/S0140-6736(20)30045-3]
2 **Yoshioka K**, Hirakawa Y, Kurano M, Ube Y, Ono Y, Kojima K, Iwama T, Kano K, Hasegawa S, Inoue T, Shimada T, Aoki J, Yatomi Y, Nangaku M, Inagi R. Lysophosphatidylcholine mediates fast decline in kidney function in diabetic kidney disease. *Kidney Int* 2022; **101**: 510-526 [PMID: 34856312 DOI: 10.1016/j.kint.2021.10.039]
3 **Wei PZ**, Szeto CC. Mitochondrial dysfunction in diabetic kidney disease. *Clin Chim Acta* 2019; **496**: 108-116 [PMID: 31276635 DOI: 10.1016/j.cca.2019.07.005]
4 **Forbes JM**, Thorburn DR. Mitochondrial dysfunction in diabetic kidney disease. *Nat Rev Nephrol* 2018; **14**: 291-312 [PMID: 29456246 DOI: 10.1038/nrneph.2018.9]
5 **Liu K**, Ji K, Guo L, Wu W, Lu H, Shan P, Yan C. Mesenchymal stem cells rescue injured endothelial cells in an *in vitro* ischemia-reperfusion model *via* tunneling nanotube like structure-mediated mitochondrial transfer. *Microvasc Res* 2014; **92**: 10-18 [PMID: 24486322 DOI: 10.1016/j.mvr.2014.01.008]
6 **Qi H**, Casalena G, Shi S, Yu L, Ebefors K, Sun Y, Zhang W, D'Agati V, Schlondorff D, Haraldsson B, Böttinger E, Daehn I. Glomerular Endothelial Mitochondrial Dysfunction Is Essential and Characteristic of Diabetic Kidney Disease

Susceptibility. *Diabetes* 2017; **66**: 763-778 [PMID: 27899487 DOI: 10.2337/db16-0695]
7 **Qi W**, Keenan HA, Li Q, Ishikado A, Kannt A, Sadowski T, Yorek MA, Wu IH, Lockhart S, Coppey LJ, Pfenninger A, Liew CW, Qiang G, Burkart AM, Hastings S, Pober D, Cahill C, Niewczas MA, Israelsen WJ, Tinsley L, Stillman IE, Amenta PS, Feener EP, Vander Heiden MG, Stanton RC, King GL. Pyruvate kinase M2 activation may protect against the progression of diabetic glomerular pathology and mitochondrial dysfunction. *Nat Med* 2017; **23**: 753-762 [PMID: 28436957 DOI: 10.1038/nm.4328]
8 **Reidy K**, Kang HM, Hostetter T, Susztak K. Molecular mechanisms of diabetic kidney disease. *J Clin Invest* 2014; **124**: 2333-2340 [PMID: 24892707 DOI: 10.1172/JCI72271]
9 **Hayakawa K**, Chan SJ, Mandeville ET, Park JH, Bruzzese M, Montaner J, Arai K, Rosell A, Lo EH. Protective Effects of Endothelial Progenitor Cell-Derived Extracellular Mitochondria in Brain Endothelium. *Stem Cells* 2018; **36**: 1404-1410 [PMID: 29781122 DOI: 10.1002/stem.2856]
10 **Feng Y**, Zhu R, Shen J, Wu J, Lu W, Zhang J, Liu K. Human Bone Marrow Mesenchymal Stem Cells Rescue Endothelial Cells Experiencing Chemotherapy Stress by Mitochondrial Transfer Via Tunneling Nanotubes. *Stem Cells Dev* 2019; **28**: 674-682 [PMID: 30808254 DOI: 10.1089/scd.2018.0248]
11 **Konari N**, Nagaishi K, Kikuchi S, Fujimiya M. Mitochondria transfer from mesenchymal stem cells structurally and functionally repairs renal proximal tubular epithelial cells in diabetic nephropathy in vivo. *Sci Rep* 2019; **9**: 5184 [PMID: 30914727 DOI: 10.1038/s41598-019-40163-y]
12 **Islam MN**, Das SR, Emin MT, Wei M, Sun L, Westphalen K, Rowlands DJ, Quadri SK, Bhattacharya S, Bhattacharya J. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med* 2012; **18**: 759-765 [PMID: 22504485 DOI: 10.1038/nm.2736]
13 **Han H**, Hu J, Yan Q, Zhu J, Zhu Z, Chen Y, Sun J, Zhang R. Bone marrow-derived mesenchymal stem cells rescue injured H9c2 cells *via* transferring intact mitochondria through tunneling nanotubes in an *in vitro* simulated ischemia/reperfusion model. *Mol Med Rep* 2016; **13**: 1517-1524 [PMID: 26718099 DOI: 10.3892/mmr.2015.4726]
14 **Zhang J**, Zhang J, Zhao L, Xin Y, Liu S, Cui W. Differential roles of microtubules in the two formation stages of membrane nanotubes between human mesenchymal stem cells and neonatal mouse cardiomyocytes. *Biochem Biophys Res Commun* 2019; **512**: 441-447 [PMID: 30904163 DOI: 10.1016/j.bbrc.2019.03.075]
15 **Fergie N**, Todd N, McClements L, McAuley D, O'Kane C, Krasnodembskaya A. Hypercapnic acidosis induces mitochondrial dysfunction and impairs the ability of mesenchymal stem cells to promote distal lung epithelial repair. *FASEB J* 2019; **33**: 5585-5598 [PMID: 30649987 DOI: 10.1096/fj.201802056R]
16 **Babenko VA**, Silachev DN, Popkov VA, Zorova LD, Pevzner IB, Plotnikov EY, Sukhikh GT, Zorov DB. Miro1 Enhances Mitochondria Transfer from Multipotent Mesenchymal Stem Cells (MMSC) to Neural Cells and Improves the Efficacy of Cell Recovery. *Molecules* 2018; **23** [PMID: 29562677 DOI: 10.3390/molecules23030687]
17 **Shanmughapriya S**, Langford D, Natarajaseenivasan K. Inter and Intracellular mitochondrial trafficking in health and disease. *Ageing Res Rev* 2020; **62**: 101128 [PMID: 32712108 DOI: 10.1016/j.arr.2020.101128]
18 **Galvan DL**, Long J, Green N, Chang BH, Lin JS, Schumacker P, Truong LD, Overbeek P, Danesh FR. Drp1S600 phosphorylation regulates mitochondrial fission and progression of nephropathy in diabetic mice. *J Clin Invest* 2019; **129**: 2807-2823 [PMID: 31063459 DOI: 10.1172/JCI127277]
19 **Gong L**, Chen B, Zhang J, Sun Y, Yuan J, Niu X, Hu G, Chen Y, Xie Z, Deng Z, Li Q, Wang Y. Human ESC-sEVs alleviate age-related bone loss by rejuvenating senescent bone marrow-derived mesenchymal stem cells. *J Extracell Vesicles* 2020; **9**: 1800971 [PMID: 32944188 DOI: 10.1080/20013078.2020.1800971]
20 **Livingston MJ**, Wang J, Zhou J, Wu G, Ganley IG, Hill JA, Yin XM, Dong Z. Clearance of damaged mitochondria *via* mitophagy is important to the protective effect of ischemic preconditioning in kidneys. *Autophagy* 2019; **15**: 2142-2162 [PMID: 31066324 DOI: 10.1080/15548627.2019.1615822]
21 **Li B**, Zheng Z, Wei Y, Wang M, Peng J, Kang T, Huang X, Xiao J, Li Y, Li Z. Therapeutic effects of neuregulin-1 in diabetic cardiomyopathy rats. *Cardiovasc Diabetol* 2011; **10**: 69 [PMID: 21798071 DOI: 10.1186/1475-2840-10-69]
22 **Bhakkiyalakshmi E**, Shalini D, Sekar TV, Rajaguru P, Paulmurugan R, Ramkumar KM. Therapeutic potential of pterostilbene against pancreatic beta-cell apoptosis mediated through Nrf2. *Br J Pharmacol* 2014; **171**: 1747-1757 [PMID: 24417315 DOI: 10.1111/bph.12577]
23 **Choi HJ**, Choi S, Kim JG, Song MH, Shim KS, Lim YM, Kim HJ, Park K, Kim SE. Enhanced tendon restoration effects of anti-inflammatory, lactoferrin-immobilized, heparin-polymeric nanoparticles in an Achilles tendinitis rat model. *Carbohydr Polym* 2020; **241**: 116284 [PMID: 32507170 DOI: 10.1016/j.carbpol.2020.116284]
24 **Wu PT**, Su WR, Li CL, Hsieh JL, Ma CH, Wu CL, Kuo LC, Jou IM, Chen SY. Inhibition of CD44 induces apoptosis, inflammation, and matrix metalloproteinase expression in tendinopathy. *J Biol Chem* 2019; **294**: 20177-20184 [PMID: 31732563 DOI: 10.1074/jbc.RA119.009675]
25 **Tang L**, Li K, Zhang Y, Li H, Li A, Xu Y, Wei B. Quercetin liposomes ameliorate streptozotocin-induced diabetic nephropathy in diabetic rats. *Sci Rep* 2020; **10**: 2440 [PMID: 32051470 DOI: 10.1038/s41598-020-59411-7]
26 **Yue T**, Xu HL, Chen PP, Zheng L, Huang Q, Sheng WS, Zhuang YD, Jiao LZ, Chi TT, ZhuGe DL, Liu JJ, Zhao YZ, Lan L. Combination of coenzyme Q10-loaded liposomes with ultrasound targeted microbubbles destruction (UTMD) for early theranostics of diabetic nephropathy. *Int J Pharm* 2017; **528**: 664-674 [PMID: 28642201 DOI: 10.1016/j.ijpharm.2017.06.070]
27 **Shiogama K**, Kitazawa K, Mizutani Y, Onouchi T, Inada K, Tsutsumi Y. New Grocott Stain without Using Chromic Acid. *Acta Histochem Cytochem* 2015; **48**: 9-14 [PMID: 25861133 DOI: 10.1267/ahc.14045]
28 **Kunter U**, Rong S, Djuric Z, Boor P, Müller-Newen G, Yu D, Floege J. Transplanted mesenchymal stem cells accelerate glomerular healing in experimental glomerulonephritis. *J Am Soc Nephrol* 2006; **17**: 2202-2212 [PMID: 16790513 DOI: 10.1681/ASN.2005080815]
29 **Almeida A**, Lira R, Oliveira M, Martins M, Azevedo Y, Silva KR, Carvalho S, Cortez E, Stumbo AC, Carvalho L, Thole A. Bone marrow-derived mesenchymal stem cells transplantation ameliorates renal injury through anti-fibrotic and anti-inflammatory effects in chronic experimental renovascular disease. *Biomed J* 2022; **45**: 629-641 [PMID: 34333108 DOI: 10.1016/j.bj.2021.07.009]

30 **Boukelmoune N**, Chiu GS, Kavelaars A, Heijnen CJ. Mitochondrial transfer from mesenchymal stem cells to neural stem cells protects against the neurotoxic effects of cisplatin. *Acta Neuropathol Commun* 2018; **6**: 139 [PMID: 30541620 DOI: 10.1186/s40478-018-0644-8]

31 **Mahrouf-Yorgov M**, Augeul L, Da Silva CC, Jourdan M, Rigolet M, Manin S, Ferrera R, Ovize M, Henry A, Guguin A, Meningaud JP, Dubois-Randé JL, Motterlini R, Foresti R, Rodriguez AM. Mesenchymal stem cells sense mitochondria released from damaged cells as danger signals to activate their rescue properties. *Cell Death Differ* 2017; **24**: 1224-1238 [PMID: 28524859 DOI: 10.1038/cdd.2017.51]

32 **Zhang Y**, Yu Z, Jiang D, Liang X, Liao S, Zhang Z, Yue W, Li X, Chiu SM, Chai YH, Liang Y, Chow Y, Han S, Xu A, Tse HF, Lian Q. iPSC-MSCs with High Intrinsic MIRO1 and Sensitivity to TNF-α Yield Efficacious Mitochondrial Transfer to Rescue Anthracycline-Induced Cardiomyopathy. *Stem Cell Reports* 2016; **7**: 749-763 [PMID: 27641650 DOI: 10.1016/j.stemcr.2016.08.009]

33 **Melcher M**, Danhauser K, Seibt A, Degistirici Ö, Baertling F, Kondadi AK, Reichert AS, Koopman WJH, Willems PHGM, Rodenburg RJ, Mayatepek E, Meisel R, Distelmaier F. Modulation of oxidative phosphorylation and redox homeostasis in mitochondrial NDUFS4 deficiency *via* mesenchymal stem cells. *Stem Cell Res Ther* 2017; **8**: 150 [PMID: 28646906 DOI: 10.1186/s13287-017-0601-7]

34 **Yang H**, Borg TK, Ma Z, Xu M, Wetzel G, Saraf LV, Markwald R, Runyan RB, Gao BZ. Biochip-based study of unidirectional mitochondrial transfer from stem cells to myocytes *via* tunneling nanotubes. *Biofabrication* 2016; **8**: 015012 [PMID: 26844857 DOI: 10.1088/1758-5090/8/1/015012]

35 **Court AC**, Le-Gatt A, Luz-Crawford P, Parra E, Aliaga-Tobar V, Bátiz LF, Contreras RA, Ortúzar MI, Kurte M, Elizondo-Vega R, Maracaja-Coutinho V, Pino-Lagos K, Figueroa FE, Khoury M. Mitochondrial transfer from MSCs to T cells induces Treg differentiation and restricts inflammatory response. *EMBO Rep* 2020; **21**: e48052 [PMID: 31984629 DOI: 10.15252/embr.201948052]

36 **Yang Y**, Ye G, Zhang YL, He HW, Yu BQ, Hong YM, You W, Li X. Transfer of mitochondria from mesenchymal stem cells derived from induced pluripotent stem cells attenuates hypoxia-ischemia-induced mitochondrial dysfunction in PC12 cells. *Neural Regen Res* 2020; **15**: 464-472 [PMID: 31571658 DOI: 10.4103/1673-5374.266058]

37 **Jiang D**, Xiong G, Feng H, Zhang Z, Chen P, Yan B, Chen L, Gandhervin K, Ma C, Li C, Han S, Zhang Y, Liao C, Lee TL, Tse HF, Fu QL, Chiu K, Lian Q. Donation of mitochondria by iPSC-derived mesenchymal stem cells protects retinal ganglion cells against mitochondrial complex I defect-induced degeneration. *Theranostics* 2019; **9**: 2395-2410 [PMID: 31149051 DOI: 10.7150/thno.29422]

38 **Sinclair KA**, Yerkovich ST, Hopkins PM, Chambers DC. Characterization of intercellular communication and mitochondrial donation by mesenchymal stromal cells derived from the human lung. *Stem Cell Res Ther* 2016; **7**: 91 [PMID: 27406134 DOI: 10.1186/s13287-016-0354-8]

39 **Martin OJ**, Lai L, Soundarapandian MM, Leone TC, Zorzano A, Keller MP, Attie AD, Muoio DM, Kelly DP. A role for peroxisome proliferator-activated receptor γ coactivator-1 in the control of mitochondrial dynamics during postnatal cardiac growth. *Circ Res* 2014; **114**: 626-636 [PMID: 24366168 DOI: 10.1161/CIRCRESAHA.114.302562]

40 **Bhargava P**, Schnellmann RG. Mitochondrial energetics in the kidney. *Nat Rev Nephrol* 2017; **13**: 629-646 [PMID: 28804120 DOI: 10.1038/nrneph.2017.107]

41 **Pal PB**, Sinha K, Sil PC. Mangiferin attenuates diabetic nephropathy by inhibiting oxidative stress mediated signaling cascade, TNFα related and mitochondrial dependent apoptotic pathways in streptozotocin-induced diabetic rats. *PLoS One* 2014; **9**: e107220 [PMID: 25233093 DOI: 10.1371/journal.pone.0107220]

42 **Xiang E**, Han B, Zhang Q, Rao W, Wang Z, Chang C, Zhang Y, Tu C, Li C, Wu D. Human umbilical cord-derived mesenchymal stem cells prevent the progression of early diabetic nephropathy through inhibiting inflammation and fibrosis. *Stem Cell Res Ther* 2020; **11**: 336 [PMID: 32746936 DOI: 10.1186/s13287-020-01852-y]

43 **Ezquer F**, Ezquer M, Simon V, Pardo F, Yañez A, Carpio D, Conget P. Endovenous administration of bone-marrow-derived multipotent mesenchymal stromal cells prevents renal failure in diabetic mice. *Biol Blood Marrow Transplant* 2009; **15**: 1354-1365 [PMID: 19822294 DOI: 10.1016/j.bbmt.2009.07.022]

44 **Zhang B**, Zhang X, Zhang C, Shen Q, Sun G, Sun X. Notoginsenoside R1 Protects db/db Mice against Diabetic Nephropathy *via* Upregulation of Nrf2-Mediated HO-1 Expression. *Molecules* 2019; **24** [PMID: 30634720 DOI: 10.3390/molecules24020247]

45 **Jourde-Chiche N**, Fakhouri F, Dou L, Bellien J, Burtey S, Frimat M, Jarrot PA, Kaplanski G, Le Quintrec M, Pernin V, Rigothier C, Sallée M, Fremeaux-Bacchi V, Guerrot D, Roumenina LT. Endothelium structure and function in kidney health and disease. *Nat Rev Nephrol* 2019; **15**: 87-108 [PMID: 30607032 DOI: 10.1038/s41581-018-0098-z]

46 **Tkach M**, Théry C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. *Cell* 2016; **164**: 1226-1232 [PMID: 26967288 DOI: 10.1016/j.cell.2016.01.043]

47 **Sun C**, Liu X, Wang B, Wang Z, Liu Y, Di C, Si J, Li H, Wu Q, Xu D, Li J, Li G, Wang Y, Wang F, Zhang H. Endocytosis-mediated mitochondrial transplantation: Transferring normal human astrocytic mitochondria into glioma cells rescues aerobic respiration and enhances radiosensitivity. *Theranostics* 2019; **9**: 3595-3607 [PMID: 31281500 DOI: 10.7150/thno.33100]

48 **Nakazaki M**, Morita T, Lankford KL, Askenase PW, Kocsis JD. Small extracellular vesicles released by infused mesenchymal stromal cells target M2 macrophages and promote TGF-β upregulation, microvascular stabilization and functional recovery in a rodent model of severe spinal cord injury. *J Extracell Vesicles* 2021; **10**: e12137 [PMID: 34478241 DOI: 10.1002/jev2.12137]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com