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Contents

Monthly Volume 16 Number 2 February 26, 2024

EDITORIAL

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

World Journal of Stem Cells

Monthly Volume 16 Number 2 February 26, 2024

Contents

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Monthly Volume 16 Number 2 February 26, 2024

Contents

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EDITORIAL

Multiple pretreatments can effectively improve the functionality of mesenchymal stem cells

Xin-Xing Wan, Xi-Min Hu, Kun Xiong

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Abstract

In this editorial, we offer our perspective on the groundbreaking study entitled "Hypoxia and inflammatory factor preconditioning enhances the immunosuppressive properties of human umbilical cord mesenchymal stem cells", recently published in *World Journal of Stem Cells*. Despite over three decades of research on the clinical application of mesenchymal stem cells (MSCs), only a few therapeutic products have made it to clinical use, due to multiple preclinical and clinical challenges yet to be addressed. The study proved the hypoxia and inflammatory factor preconditioning led to higher immunosuppressive effects of MSCs without damaging their biological characteristics, which revealed the combination of inflammatory factors and hypoxic preconditioning offers a promising approach to enhance the function of MSCs. As we delve deeper into the intricacies of pretreatment methodologies, we anticipate a transformative shift in the landscape of MSC-based therapies, ultimately contributing to improved patient outcomes and advancing the field as a whole.

Key Words: Mesenchymal stem cells; Inflammatory factor; Hypoxia; Pretreatment

Core Tip: We offer our perspective on the groundbreaking study titled "Hypoxia and inflammatory factor preconditioning enhances the immunosuppressive properties of human umbilical cord mesenchymal stem cells", and recently published in the *World Journal of Stem Cells*.

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INTRODUCTION

Stem cells (SCs) are characterized by multipotency, asymmetric division and having the capacity of self-renewal[1-3]. SCs can directly or indirectly stimulate resident cells, regulate inflammation, release biological molecules and remodel extracellular matrix (ECM) to benefit various diseases[4-8]. SC can be classified into totipotent, pluripotent and monopotent SCs according the different differentiation potentials. There is now a consensus that the clinical use of SC holds great promise for the treatment of a wide range of intractable diseases. As a result, there are various clinical trials to explore the clinical use of SC in many diseases. Mesenchymal SCs (MSCs) are multipotent stromal cells, which have good self-renewal and pluripotent differentiation potentials. The potential for MSCs as a cell-based therapy in treating moderate immunologic disorders and regeneration has been well clarified, and MSCs are the most chosen SCs in clinical treatment[9]. MSCs can be derived from various tissues, including bone marrow, adipose tissue, Wharton's Jell, dental pulp, menstrual blood and umbilical cord blood gleaned efficiently[10,11]. Although MSCs derived from different tissues share the same basic functional characteristics, there are still differences in their functional strengths, such as cell size, proliferative potential, secreted cytokines, and immunosuppression. MSCs can change the micro-environment in the tissues and increase cell differentiation and regeneration ability, the function of MSCs in the treatment of immunologic disorders has been well established. So, MSCs are used clinically to try to treat many diseases including vascular diseases, immune diseases and wounds.

SCOPE AND BENEFITS OF MSCS IN CLINICAL UTILIZATION

MSCs have emerged as a prominent cell source for SC therapy, owing to their renewable nature, immunomodulatory properties, minimal risk of tumorigenesis and lack of ethical constraints[12]. Many subtypes of MSCs, such as adipose-derived MSCs, human umbilical MSCs (HUMSCs), and bone marrow MSCs are eligible for clinical use, without significant functional abnormalities or side effects[11,13,14]. MSCs are primary plastic adherent cells with immense proliferative potential and the ability to self-renew and differentiate. These cells can be easily cultured *in vitro*, and MSCs do not express CD45, CD34, CD14, CD11b, CD79a, CD19, or major histocompatibility complex (MHC) class II, which can curtail immune rejection and facilitate clinical transplantation of MSCs[15-17]. MSCs are widely employed in traumatic tissue repair, immune disorders, and cancer therapy. The proliferative capacity and immunogenicity of MSCs from different human populations vary greatly, and the osteogenic potential of MSCs derived from diabetic patients is decreased than health volunteers[18], while MSCs isolated from patients with autoimmune diseases have morphological and some functional abnormalities[19], which implies that the normal MSCs transplants may restore the MSCs function and alleviate the disease.

DEFICIENCIES OF MSCS IN CLINICAL USE AND APPROACHES TO IMPROVEMENT

The clinical application of MSCs has been conducted for more than thirty years[15,20], and there are thousands of clinical trials are currently being conducted (http://clinicaltrials.gov). However, only a few MSC-based therapeutic products have been approved for clinical use. There are still many key preclinical and clinical challenges to be solved, which are mainly in the areas of cell preparation methods, consistency, efficiency, reproducibility, processing time, scalability and purity of MSCs. So future investigations into the pretreatment of MSCs have the potential to yield improved outcomes and facilitate the implementation of clinical MSCs.

MSCs release a complex array of active ingredients that are influenced by the host microenvironment (inflammatory state, hypoxia, and ECM), resulting in highly variable factors that shape their distinct functions[15,21]. To achieve superior therapeutic effects in clinical applications, researchers have made advancements in treating MSCs using various methods, including genetic engineering, SC conjugation, drug or cytokine interventions, and so on. For examples, pretreatment of 3-methyladenin could inhibit autophagy in MSCs[22]; platelet derived growth factor BB (PDGF-BB) pretreatment could promote MSC migration and inhibit hydrogen peroxide induced MSC apoptosis *via* PI3K/Akt pathway[23]. The MSCs extracted from nlrp3-KO mice promoted osteogenic differentiation without affecting the phenotypes of MSC[24]. In the diabetic retinopathy rat model, the retinal vessel formation, retinal function and uveitis

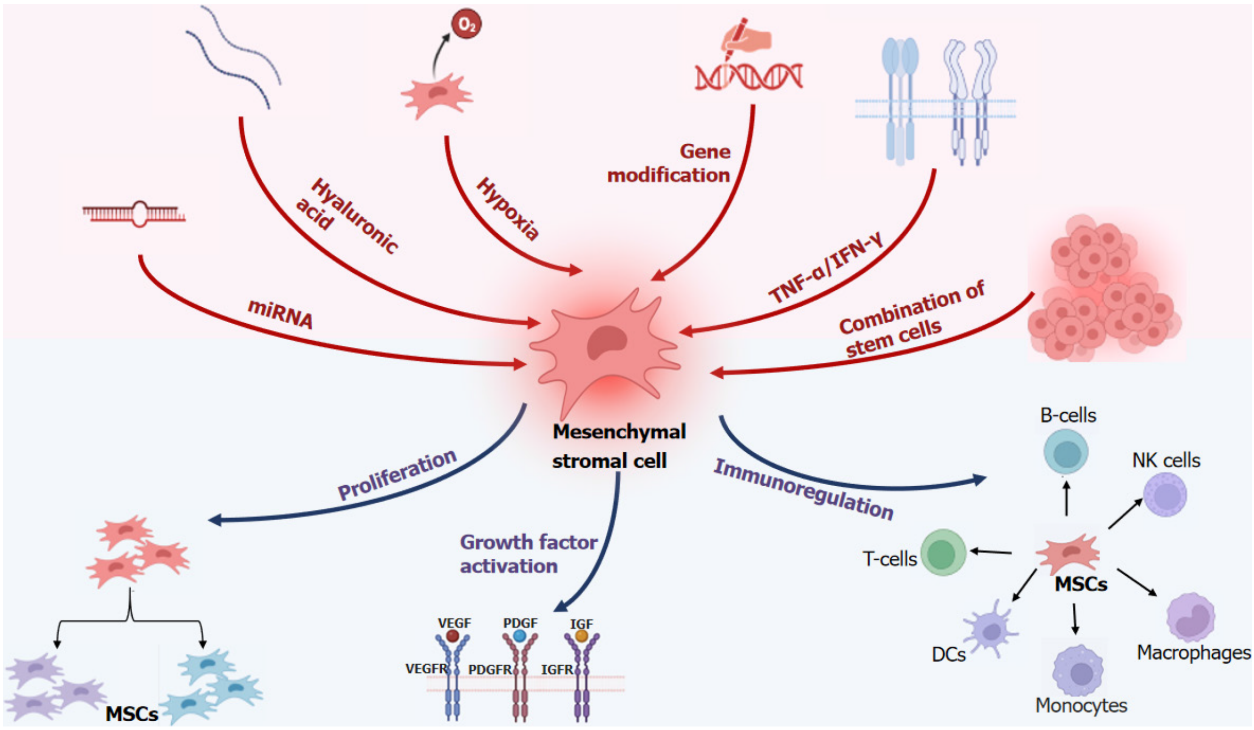

Figure 1 Multiple treatments to promote the function of mesenchymal stem cells. To enhance the function of mesenchymal stem cells (MSCs) such as proliferation, immunoregulation and the secretion of growth factors, various method such as microRNA and special gene transfection, combination of other stem cells and the treatment of hypoxia/inflammation factors *etc.* have been tried to use in the MSCs. miRNA: MicroRNA; TNF: Tumor necrosis factor; IFN-γ: interferon-γ; MSCs: Mesenchymal stem cells; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; PDGF: Platelet derived growth factor; PDGFR: platelet derived growth factor receptor; IGF: Insulin-like growth factor; IGFR: Insulin-like growth factor receptor; DCs: Dendritic cells; NK: Natural killer.

was found improved when transplanted with tacrolimus-pretreated MSCs[25]. These innovations provide new and exciting options for the clinical application of MSCs (Figure 1).

Previously, our laboratory attempted various methods to enhance the function of MSCs. One possible approach would be the overexpression of the c-jun plasmid in HUMSCs to expedite wound healing by amplifying PDGFA and hepatocyte growth factor levels in diabetic rats' wound tissues, thereby fostering angiogenesis and re-epithelialization at the wound bed[26]. Another possible solution would be the coating MSCs with ECM, which we demonstrated, could promote wound healing in diabetic rats by stimulating vascular endothelial growth factor-α, PDGF, and epidermal growth factor expression[27]. Additionally, we compared the healing rates of multiple separate wounds in diabetic foot patients injected with MSCs combined with endothelial colony-forming cells (ECFCs) and hyaluronic acid. The self-controlled wounds were only treated with conventional therapy and covered by hydrocolloid dressings, and our findings demonstrated that the combination of umbilical cord MSCs, ECFCs, and hyaluronic acid can safely accelerate the healing of refractory diabetic foot ulcers[28].

Now it has been demonstrated that laser pretreatment can also enhance the function of MSCs. Chen *et al*[29] proved low-level controllable blue light emitting diodes irradiation can enhance the activity of intracellular calcium levels to enhance the osteogenic differentiation of MSCs derived from human dental pulp. Wen *et al*[30] encapsulated MSCs in the Prussian blue nanoparticles and methacrylated gelatin hydrogels, and MSCs complex was exposed to a 1.0 W/cm^2 of 808 nm laser for 10 min for the implantation. The laser pre-treatment could improve the viability of MSCs and accelerate the regeneration of muscle. Wang *et al*[31] found low-level laser irradiation treatment of HUMSCs could significantly increase the erythrocyte count and number of myelopoiesis clones. All these results indicate that laser pretreatment could be a new strategy to further enhance the clinical use of MSCs.

Generally, there is no academic consensus on the outcomes of hypoxic intervention in MSCs. Numerous studies have shown that MSCs cultured continuously under low oxygen (1%-5%) conditions typically exhibit enhanced proliferative potential and well-maintained stemness of MSCs[32,33]. Hypoxia significantly induces MSCs to secrete angiogenic and anti-inflammatory cytokines in large amounts and enhances the migratory capacity of MSCs[32]. However, a number of experiments have also demonstrated that the apoptosis rate of MSCs increased in hypoxic environments, suggesting that the effect of hypoxia on MSCs needs further investigation[34]. Interferon-γ (IFN-γ) enhances the anti-inflammatory and therapeutic fibrotic properties of MSCs and promotes their survival[35]; theoretically, the combination of IFN-γ and hypoxic culture could enhance the therapeutic function of MSCs.

The immunocompatibility between donors and recipients is a key factor in reducing the risk of immune rejection, but it is influenced by environmental inflammatory molecules, which can induce a significant expression of MHC-II in MSCs. Further reducing the immunogenicity of MSCs is another important way to improve the efficacy of MSCs. It's evident that IFN-γ and IFN-γ/transforming growth factor β1 licensing enhance the immunomodulatory effect of MSCs on T cell proliferation[35]. Tumor necrosis factor-α preconditioning can improve the therapeutic efficacy of MSCs in atherosclerosis

[36]. In the study of "Hypoxia and inflammatory factor preconditioning enhances the immunosuppressive properties of human umbilical cord MSCs", the researchers pretreated UC-MSCs with hypoxia (2% O_2) exposure and inflammatory factors (interleukin-1β, tumor necrosis factor-α, IFN-γ)[37]. After 24 h of exposure, they found that the pretreatment caused UC-MSCs to become elongated without affecting viability, proliferation, or size. Additionally, they demonstrated that this pretreatment enhanced the expression of genes and proteins related to immune regulation and increased peripheral blood mononuclear cell and natural killer (NK) cell proliferation rates while inhibiting NK cell-induced toxicity to varying degrees. With detailed experimental data, this article further revealed that hypoxia combined with inflammatory factor preconditioning can enhance the function of MSCs, providing a new scheme for the clinical application of MSCs.

CONCLUSION

The combination of inflammatory factors and hypoxic preconditioning is an effective approach to enhance the function of MSCs. The concentration of hypoxia, the type and concentration of inflammatory factors, and the duration of preconditioning have a significant impact on MSC's function. In-depth investigations in this domain will yield improved outcomes and facilitate the utilization of clinical MSCs.

FOOTNOTES

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