Free Radical Biology and Medicine

Volume 159, 1 November 2020, Pages 164-176

Original article

Hypoxic preconditioning combined with curcumin promotes cell survival and mitochondrial quality of bone marrow mesenchymal stem cells, and accelerates cutaneous wound healing via PGC-1α/SIRT3/HIF-1α signaling

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Highlights

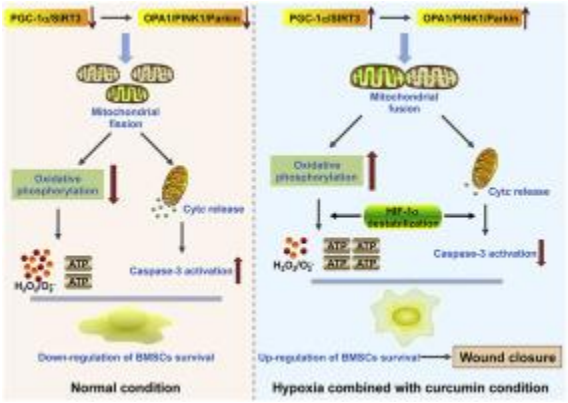
- Hypoxic preconditioning/curcumin promotes BMSCs survival and mitochondrial fusion.
- Hypoxic preconditioning/curcumin represses $H_2O_2/O_2^{\bullet-}$ production and cell apoptosis.
- HIF-1α destabilization contributes to the protective effects of curcumin.
- PGC-1α and SIRT3 are essential for hypoxia-mediated mitochondrial quality control.
- Hypoxic preconditioning/curcumin-treated BMSCs accelerate wound healing *in vivo*.

Abstract

Restrained survival and function of relocated bone marrow mesenchymal stem cells (BMSCs) is a major impediment to BMSCs-mediated tissue repair. Accumulating evidences have indicated that hypoxic preconditioning of BMSCs could enhance BMSCs' adaptability after transplantation and thus improve their therapeutic properties. Curcumin, a natural dietary product, is known to exert profound protective effects on various cellular processes. Here we showed that mild hypoxic preconditioning combined with curcumin significantly increased cell survival, enriched more cells in G2/M and S phase, and improved mitochondrial function in BMSCs. Meanwhile, hypoxic preconditioning combined with curcumin altered mitochondrial cristae shape and strongly inhibited mitochondrial cytochrome *c* release, which consequently suppressed an apoptosis signal as revealed by reduced caspase-3 cleavage in BMSCs. Moreover, hypoxic preconditioning remarkably promoted mitochondrial quality via increasing mitochondrial fusion and elevating the activity of oxidative phosphorylation (OXPHOS) and mitochondrial complex Ⅰ enzyme in BMSCs, which were in accordance with the up-regulated expression of OPA1, PINK1 and Parkin. At the mechanistic level, the destabilization of HIF-1α and the up-regulated expression of PGC-1α and SIRT3

synergistically contributed to the protective effects of hypoxic preconditioning combined with curcumin in BMSCs. The proteasome inhibitor MG132 stabilized HIF-1a expression, but not PGC-1α or SIRT3, and dramatically restrained BMSCs survival under hypoxia combined with curcumin condition. MG132 also increased mitochondrial superoxide and intracellular hydrogen peroxide (H_2O_2) production and caspase-3 activation in hypoxia combined with curcumin-treated BMSCs. Furthermore, knockdown of SIRT3 and PGC-1α by RNAi both led to caspase-3 activation in BMSCs after hypoxia and curcumin treatment. Notably, SIRT3 RNAi suppressed OXPHOS activity, while PGC-1α RNAi triggered mitochondrial superoxide and intracellular H_2O_2 production in hypoxia combined with curcumin-treated BMSCs. Finally, we showed that hypoxia combined with curcumin-treated BMSCs accelerated the cutaneous wound healing process in a mice wound model. Overall, this study suggests that hypoxic preconditioning combined with curcumin could serve as an attractive strategy for facilitating BMSCs-mediated tissue repair, and further sheds new light on the rich repertoire of PGC-1α/SIRT3/HIF-1α signaling involved in the regulation of mitochondrial quality and function for cellular adaption to hypoxia.

Graphical abstract



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Introduction

Delayed wound healing, a common complication in burns and trauma, afflicts a wide range of patients worldwide [1,2]. Successful therapeutic strategies that allow more rapid re-epithelialization would be of tremendous benefit, reducing excessive inflammatory response and suppressing the harmful aberrant collagen fiber arrangement that can occur [[3], [4], [5]]. In this regard, growth factors, genetic modifications, as well as stem cell therapy, have been used to expedite wound healing [6,7].

Compelling evidences are accumulating for the favorable effects of bone marrow mesenchymal stem cells (BMSCs) in tissue repair in terms of their self-renewal capability, multipotency, paracrine production, as well as less ethical limitations [[8], [9], [10]]. Investigational BMSCs-based therapies for wound healing have been recently introduced into clinical trials [11,12]. However, mixed responses were generated among different clinical settings, which might be ascribed, at least partially to the restricted survival of donor BMSCs [13]. The hostile disease microenvironment in the wounds leads to impaired cell function and increased cell apoptosis. Moreover, massive donor cell death exacerbates the functional damage of other tissue repair cells such as fibroblasts and keratinocytes, and thus introduces an additional burden to the wounds [14]. Therefore, there is a strong need for additional insights aimed at addressing the optimization of BMSCs tolerance properties after transplantation.

The severe hypoxic condition in injured tissue is a major obstacle for the application of BMSCs which are cultured under normal oxygen tension [15]. Once localized to the ischemic tissue, BMSCs encounter extremely low oxygen tension, which often results in cell apoptosis. The oxygen concentration in the bone marrow is 2%–7% under physiological conditions [16]. It is a critical theme that preconditioning BMSCs in sublethal hypoxic conditions for a period of time before relocating them to ischemia injury sites could enhance the cellular adaptive responses of BMSCs, such as through up-regulating anti-apoptotic genes and reducing caspase-3 activity [17,18]. Most importantly, hypoxic preconditioning of BMSCs has been shown to favor cell survival and inhibit extensive cell apoptosis, and therefore improve the therapeutic potential of BMSCs [16,19]. Toward this end, an exploration of the influence of a controlled hypoxic environment on the therapeutic properties of BMSCs is worthwhile, and the involved signaling events also need to be investigated.

Mitochondria, the cellular powerhouses, utilize most of oxygen to produce adenosine triphosphate (ATP) via electron transfer coupled with oxidative phosphorylation in mitochondrial matrix. Mitochondria also serve as important signaling organelles in response to cellular demands and environmental imperatives, including hypoxic stimulation [20,21]. Additionally, mitochondria undergo rapid dynamic fission and fusion cycle to ensure mitochondrial integrity, and maintain mitochondrial quality and function under stressful conditions [22,23]. As a matter of fact, disrupted mitochondrial integrity leads to pro-apoptotic factors release such as cytochrome *c*, and triggers mitochondria-dependent apoptosis [24,25]. Recent studies have shown that regularly shaped mitochondrial cristae facilitates the formation of respiratory chain supercomlexes and promotes oxidative phosphorylation [[26], [27], [28]]. Notably, mitochondrial fusion coordinates apoptotic cristae remodeling and cytochrome *c* release to cytoplasm, and restores mitochondrial function in many disease settings, offering a mechanism for cellular protection. Thus, modulating the mitochondrial quality may directly contribute to the improved survival of transplanted BMSCs.

Hypoxia-inducible factor-1 alpha (HIF-1α) is known to be a master regulator for governing cellular hypoxic adaption to dictate cell fate. A well-known facet of HIF-1α biology is its activation by, and modulation of hypoxia [29]. Under hypoxia, HIF-1α acts the role of promoting or resisting cell survival, which appears to be largely context-dependent [30,31]. Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) belongs to a small family of transcriptional regulators, which controls the expression of genes involved in mitochondrial biogenesis and oxidative metabolism [32]. Silent mating-type information regulation 2 homolog 3 (SIRT3) is a crucial member of the sirtuin family of nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylases. As a downstream target gene of PGC-1α, SIRT3 is typically localized in mitochondria, and integrates its role in regulating mitochondrial function related to metabolic enzyme activity, oxidative phosphorylation and anti-oxidant machinery [33]. Given the crucial roles of PGC-1α and SIRT3 in modulating mitochondrial function and oxidative metabolic programs, we hypothesized that they are likely to be involved in the regulation of cellular adaption to hypoxia.

Curcumin, best known as an anti-oxidant, is a commonly used spice and coloring agent isolated from *Curcuma longa*. Extensive researches have clearly shown that curcumin exerts diverse therapeutic effects including anti-oxidative, anti-apoptotic, anti-inflammatory, anti-diabetic and so on, which most likely attribute to its pleiotropic signaling machinery via modulating the activation of transcriptional factors and the expression of cell survival proteins [34,35]. Various preclinical cell culture and animal studies suggest that curcumin has the potential to serve as a therapeutic agent in wound healing, diabetes, and arthritis [[36], [37], [38]]. Our previous work has revealed the functional role of curcumin in facilitating BMSCs survival under hypoxia/reoxygenation-induced oxidative stress via improved mitochondrial function and alterations in signal targets [31]. Thus, based on the important literatures and our recent study, we were eager to know whether and how hypoxic preconditioning

combined with curcumin could produce synergistically protective effects on BMSCs' bioactivity and thus potentiate their therapeutic properties *in vivo*.

In this study, we demonstrated that a selective physiological preconditioning of 5% O_2 for 24 h in combination with curcumin significantly promoted cell survival and mitochondrial quality of BMSCs, and enhanced BMSCs' therapeutic effects on cutaneous wound healing in mice via the modulation of PGC-1α/SIRT3/HIF-1α signaling.

Section snippets

Ethics statement

The animal study was approved by the Institutional Animal Care and Use Committee of Fourth Military Medical University. All animal experiments were conducted in compliance with the approved guidelines where applicable [39] and every effort was made to minimize animals suffering. Surgeries were performed under isoflurane inhalation anesthesia.

Cell culture and hypoxic treatment

Rat BMSCs were obtained from Cyagen Biosciences Company (Guangzhou, China) and cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12

The characterization of curcumin-pretreated BMSCs under hypoxic condition

To determine the optimal duration of hypoxic culture for BMSCs, cells were cultured at 5% O_2 for selective time episodes (12, 24, 36, 48 and 72 h). The Image-iT Green Hypoxia Reagent was applied to detect the hypoxic condition (Fig. 1A). After different periods of hypoxia, we found that BMSCs survival peaked at 24 h of hypoxic exposure, with the highest survival rate observed in hypoxia combined with curcumin-treated BMSCs (Fig. 1B). The cell cycle stages of differently treated BMSCs were

Discussion

BMSCs transplantation has been increasingly indicated as a desirable approach for improving tissue repair [4]. Although BMSCs are known to produce concerted therapeutic effects by cell replacement and by cell empowerment, the reported therapeutic effects are rather restricted, in large part due to the attenuated cell viability and function of relocated BMSCs [50]. To solve this problem, various strategies are designed to increase the biological activity of implanted BMSCs in injured tissue. Of

Declaration of competing interest

The authors declare no relevant competing financial interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China [81901967, 81530064], Shaanxi Province Natural Science Basic Research Program [2019JQ-478], and Subject Booster Program of Xijing Hospital [XJZT18MJ38].

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