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Extended Abstract
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Association of apolipoprotein E polymorphism with maximal oxygen uptake after exercise training: A Study of Chinese young adult

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Introduction

Mesenchymal stem cell (MSC)-based therapies have had positive outcomes both in animal models of cardiovascular diseases and in clinical patients. However, the number and function of MSCs decline during hypoxia and serum deprivation (H/SD), reducing their ability to contribute to endogenous injury repair. MicroRNA-34a (miR-34a) is originally identified as a TP53-targeted miRNA that modulates cell functions, including apoptosis, proliferation, and senescence via several signaling pathways, and hence is an appealing target for MSC-based therapy for myocardial infarction.

Methods

Bone marrow-derived MSCs were isolated from 60–80 g male donor rats. Expression levels of miR-34a were determined by qRT-PCR. The roles of miR-34a in regulating cell vitality, apoptosis and senescence were investigated using the cell counting kit (CCK-8) assay, flow cytometric analysis of Annexin V-FITC/PI staining and senescence-associated β-galactosidase (SA-β-gal) staining, respectively. The expression of silent information regulator 1 (SIRT1) and forkhead box class O 3a (FOXO3a) and of apoptosis- and senescence-associated proteins in MSCs were analyzed by western blotting.

Results

The results of the current study showed that miR-34a was significantly up-regulated under H/SD conditions in MSCs, while overexpression of miR-34a was significantly associated with increased apoptosis, impaired cell vitality and aggravated senescence. Moreover, we found that the mechanism underlying the proapoptotic function of miR-34a involves activation of the SIRT1/FOXO3a pathway, mitochondrial dysfunction and finally, activation of the intrinsic apoptosis pathway. Further study showed that miR-34a can also aggravate MSC senescence, an effect which was partly abolished by the reactive oxygen species (ROS) scavenger, N-acetylcysteine (NAC).

Conclusions

Our study demonstrates for the first time that miR-34a plays pro-apoptotic and pro-senescence roles in MSCs by targeting SIRT1. Thus, inhibition of miR-34a might have important therapeutic implications in MSC-based therapy for myocardial infarction.

Introduction

Ischemic heart disease (IHD) is the leading cause of death worldwide, and the resulting heart failure aggravates a country's health burden, particularly in developed countries. Existing therapies are typically only able to slow, rather than reverse or prevent, the progression of heart failure. Furthermore, side effects remain the key issue among these effective therapeutics. In the last few years, bone marrow-derived mesenchymal stem cells (MSCs) have been found to function as one of the most suitable candidate seed cells for repairing and regenerating cardiomyocytes as well as restoring heart function, and have been widely studied. Transplantation of MSCs leads to improved neovascularization of ischemic myocardium and inhibition of myocardial fibrosis, in addition to an increase in the secretion of prosurvival growth factors, including vascular endothelial growth factor, insulin-like growth factor, and hepatocyte growth factor. Despite these advantages, the poor survival rate of MSCs within the first few days after engrafting in infarcted hearts leads to only marginal functional improvement. The harsh microenvironment of the infarcted myocardium produces high levels of oxidative stress, which makes a great contribution to cellular senescence and causes a sharp decline in the proliferative capacity and regenerative potential of MSCs. There is thus an urgent need to identify a strategy to protect the cells against the hostile microenvironment created by ischemia, hypoxia, the inflammatory response, and pro-apoptotic and pro-senescence factors in order to improve the efficacy of MSC transplantation therapy.

MicroRNAs (miRNAs) are endogenous ~22-nucleotide RNAs that have emerged as negative regulators of

gene expression, acting by targeting mRNAs for cleavage or translational repression, which occurs primarily through base pairing to the 3′ untranslated regions (UTRs) of target mRNAs. With rapid advances in understanding of the regulation and roles of these small, noncoding RNAs in cardiac pathology, the therapeutic potential of regulation of miRNAs in cardiac disease settings is considered high. Among the known miRNAs, expression of miR-34a was found to be elevated in mouse hearts after myocardial infarction (MI) and in cardiac tissue from patients with heart disease, while inhibition of the expression of miR-34a alleviated apoptosis and senescence in myocardial cells and other cell lines. However, the precise role of miR-34a in MSCs has not been unraveled to date.

Silent information regulator 1 (SIRT1), one of the potential targets of miR-34a, is an NAD-dependent deacetylase that regulates apoptosis in response to oxidative and genotoxic stress and plays a critical role in regulating cell cycle, senescence, and metabolism. Initially identified as a longevity gene, SIRT1 has recently been implicated as a novel modulator of myocyte homeostasis, playing a key role in cardiomyopathy through the deacetylation of forkhead box O transcription factor 3a (FOXO3a), which was also acknowledged as the transcription factor most closely related to the anti-oxidative protective effects associated with longevity. Further study showed in endothelial progenitor cells (EPCs) that SIRT1 has a pivotally protective role in the regulation of EPC apoptosis induced by H_2O_2, and that SIRT1 exerted this protective effect by inhibiting FOXO3a via FOXO3a ubiquitination and subsequent degradation. However, it is entirely unknown whether SIRT1 affects biological activities in MSCs; and if so, what role FOXO3a plays in this process.

In the current study, we tested the hypothesis that overexpression of miR-34a increases cellular susceptibility to hypoxia and serum deprivation (H/SD)-induced apoptosis and aggravates cell senescence, and investigated the underlying mechanisms. The results showed that miR-34a played a crucial role in a plethora of biological processes via regulation of SIRT1/FOXO3a and the reactive oxygen species (ROS) pathway in MSCs. Inhibition of miR-34a might therefore be a promising therapeutic strategy for enhancing the biological functions of MSCs, thus demonstrating great therapeutic potential in clinical transplantation.

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