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MicroRNA-23a is involved in tumor necrosis factor-α induced apoptosis in mesenchymal stem cells and myocardial infarction

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ABSTRACT

Cell therapy has emerged as an attractive therapeutic modality to treat myocardial infarction (MI) via repairing damaged myocardium, and mesenchymal stem cells (MSCs) are an appealing therapeutic approach for cardiac regeneration. However, the clinical application of MSC-based therapy is restricted because of the poor survival of implanted cells, and this poor survival remains poorly understood. Using a tumor necrosis factor (TNF)-α-induced bone marrow (BM)-MSC injury model in vitro and a rat MI model in vivo, we showed in the current study that miR-23a was involved in TNF-α-induced BM-MSC apoptosis through regulating caspase-7 and that the injection of BM-MSCs overexpressing miR-23a could improve left ventricular (LV) function and reduce infarct size in the rat MI model. Our findings elucidate the etiology of MI and provide an alternative treatment strategy for patients with heart failure caused by MI who are not optimal candidates for surgical treatment.

Introduction

Myocardial infarction (MI) is a common presentation of ischemic heart disease and results from the interruption of the blood supply to part of the heart, causing heart cells to die. According to the WHO in 2004, ischemic heart disease accounted for an estimated 12.2% of worldwide deaths, and this percentage continues to increase (Valensi et al., 2011).

Recently, cell therapy has emerged as an attractive therapeutic modality to treat MI via repairing damaged myocardium. Several types of cells, including hematopoietic stem cells (HSCs) (Jackson et al., 2001), bone marrow-derived mesenchymal stem cells (BM-MSCs) (Orlic et al., 2001; Strauer et al., 2002), and endothelial progenitors (Cornel Badorff et al., 2003; Stefan Rupp et al., 2004), can differentiate into cardiomyocytes both in vitro and in vivo. Of these cells, MSCs have been extensively investigated as an appealing therapeutic approach for cardiac regeneration because of their distinctive characteristics properties, such as the ease of their acquisition and isolation, their high expansion capability in culture, and their low immunogenicity (Kim et al., 2009). However, the clinical application of MSC-based therapy is restricted, mainly because of the poor survival of implanted cells (Song et al., 2010). For example, evidence from a swine model of MI suggested that only 5% of grafted MSCs survive for 2 weeks in the infarcted myocardium (Freyman et al., 2006). A similarly low survival rate of implanted MSCs was also observed in a mouse mode of Mil (Toma et al., 2002). Meanwhile, another reason for the low number of injected cells in the myocardium is supposed to be the poor cell retention, and migration of cells into remote organs (Gyongyosi et al., 2008). Thus, viability seems to pose a major obstacle for MSCs and possibly any cell-based therapeutic strategy in the infarcted heart. In addition, environmental factors may further exacerbate the damage caused by infarction (Mylotte et al., 2008). The production of oxygen-derived free radicals and inflammatory cytokines triggered by myocardial injury probably stimulates cell death and initiates apoptosis, and the ischemic conditions, which lead to a lack of nutrients and oxygen, may affect the viability of the grafted cells as well (Frangogiannis, 2006). Therefore, the major factors that influence the apoptosis of implanted MSCs must be identified.

TNF-α, which is mainly produced by activated macrophages, is the major extrinsic mediator of apoptosis (Locksley et al., 2001). Tumor necrosis factor (TNF)-α converts the interferon (IFN)-γ-activated, non-apoptotic form of TNF receptor superfamily member 6 (Fas) to a caspase-3- and caspase-8-associated proapoptotic cascade by inhibiting nuclear factor κB (NF-κB), which results in apoptosis of BM-MSCs (Liu et al., 2011). Indeed, TNF-α-induced cell injury is a widely accepted cellular model for investigating apoptotic mechanisms, and many specific

Abbreviations: MI, myocardial infarction; MSCs, mesenchymal stem cells; BM, bone marrow; HSCs, hematopoietic stem cells; NF-κB, nuclear factor κB; TNF, tumor necrosis factor; miRNAs, microRNAs; SD, serum deprivation; TUNEL, terminal deoxynucleotide transferase dUTP nick-end labeling; LAD, left anterior descending artery; LV, left ventricular; LVDS, LV end-systolic diameter; LVDD, LV end-diastolic diameter; EF, ejecting fraction; FS, fractional shortening; IVS, inter-ventricular septum thickness; AAR, area at risk; TTC, triphenyltetrazolium chloride; ESD, end-systolic dimension; EDD, end-diastolic dimension.

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downstream players have been identified based on this model (Fujikura et al., 2012; He and Ting, 2002).

MicroRNAs (miRNAs) have emerged as key regulators in diverse biological pathways, including apoptosis (Pasquinelli, 2012; Pritchard et al., 2012). Given their diverse roles, it is not surprising that disrupting the functions of miRNAs contributes to many human diseases, including cancer, neurological diseases, and heart conditions (Bartel, 2009; Sayed and Abdellatif, 2011). Additionally, recent studies have shown that miRNAs are the key modulators in the fate determination of stem cells (Houbaviy et al., 2003; Suh et al., 2004). Altered miRNA profiles are observed during MSC apoptosis induced by hypoxia and serum deprivation (SD), and the overexpressions of miR-21, miR-23a, and miR-20 promote the survival of MSCs exposed to hypoxia/SD (Nie et al., 2011). Previously, Ruan et al. (2012) demonstrated the effects of the down-regulation of miR-23a on TNF-α-induced endothelial cell apoptosis, indicating the importance of miRNAs on the TNF-related apoptosis signaling pathway and even tissue regeneration. In addition, the mechanisms of TNF-α-induced endothelial cell injury have been extensively explored (Martin-Ventura et al., 2007; Zauli and Secchiero, 2006). However, BM-MSCs, as a more attractive source of cell therapy, little is known about how miRNAs influence their apoptosis.

In this study, we explored the potential effects of miRNAs on BM-MSC apoptosis and subsequent mechanisms using a TNF-α-induced BM-MSC injury model in vitro and a rat myocardial infarction model in vivo. We aimed to elucidate the detailed mechanisms that hinder the successful clinical applications of MSCs in treating MI, which may be of great importance to better understand MI and advance clinical therapy.

Materials and methods

Experimental animals

Male SD rats weighing 240 g to 280 g were used in this study. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, and all protocols were approved by the Animal Care and Use Committee of the First Hospital of Shanghai Jiaotong University School of Medicine.

Preparation of MSCs

Bone marrow cells were obtained by flushing the femurs and tibias of the rats with 10 mL of DMEM (Gibco-BRL, NY, USA) supplemented with 10% FBS and 50 IU/mL penicillin–streptomycin. Mononuclear cells were isolated via density-gradient centrifugation and transferred to culture flasks. Non-adherent cells were removed by subsequent medium changes, and MSCs were cultured and expanded until passage 3 for use.

Measurement of TNF-α-induced MSC apoptosis

Apoptosis was measured by the terminal deoxynucleotide transferase dUTP nick-end labeling (TUNEL) staining assay (Gavrieli et al., 1992). Briefly, MSCs cultured in 5% FBS were treated with either vehicle or increasing concentrations of TNF-α for 24 h. Then, the TUNEL staining assay was performed using a commercial cell death detection kit (Roche) according to the manufacturer's instructions. The number of TUNEL-positive cells was counted under a fluorescence microscope. Chromosomal condensation was estimated using the chromatin dye Hoechst 33342 (Sigma).

Detection of RNA levels by qRT-PCR

In brief, total RNA was extracted from approximately 10^5 MSCs using TRIzol reagent (Invitrogen, Carlsbad, CA), and qRT-PCR was performed on cDNA generated from 50 ng of total RNA according to the manufacturer's instructions. U6 was used as an internal control for miRNA template normalization.

Lentiviral vector construction and transduction

For lentivirus construction, the precursor sequence for miR-23a (Lenti-miR-23a), self-complementary miR-23a (Lenti-anti-miR-23a) and irrelevant sequence (negative control) were inserted into hU6-MCS-PGK-EGFP lentiviral vectors (Hanbio, Shanghai, China). The recombinant lentivirus was produced by co-transfection of 293 T cells with plasmids PSPAX2 and PMD2G with LipoFiter™ (Hanbio, Shanghai, China). Lentivirus-containing supernatant was harvested 48 h after transfection and filtered through 0.22-μm cellulose acetate filters (Millipore, USA). Recombinant lentiviruses were concentrated by ultracentrifugation (2 h at 50,000 ×*g*).

For lentivirus infection, when the BMSCs were at ~60% confluence, the culture medium was removed before infection. The cells were washed gently with PBS and treated with the virus-containing medium (MOI of approximately 10). After 24 h, culture medium was removed and fresh medium was added to the BMSCs. It was found that most of the BMSCs (>80%) expressed EGFP 48 h after the transfection. Three days after viral infection, the infection efficiency was determined by flow cytometry.

Luciferase assay

The 3′-UTR of human caspase-7 was amplified from human genomic DNA and cloned into psiCheck2 vector (Promega) by directional cloning. To generate the caspase-7 3′-UTR-Mut construct, seed regions were mutated from AAUGUGA to UCGACUC, removing all nucleotides complementary to nucleotides 2–8 of miR-23a (QuikChange XL Mutagenesis Kit; Stratagene, La Jolla, CA). HEK293 cells were seeded at a density of 50% in 6-well plates 12 h prior to transfection. HEK-293 cells were cotransfected with 3 μg of firefly luciferase reporter vector using Lipofectamine 2000. Moreover, 3 μg of miR-23a expression vector or the corresponding empty vector was cotransfected with the reporter constructs. Luciferase activity was measured 36 h after transfection. Renilla luciferase activity was normalized to firefly luciferase activity.

Western blot analysis

Proteins isolated from cultured MSCs were analyzed by western blot analysis. Equal amounts of protein were subjected to SDS-PAGE. A standard western blot analysis was performed using the Caspase-7 antibody (1:1000 dilution; Cell Signaling). GAPDH (1:5000 dilution; Cell Signaling) was used as a loading control.

The rat myocardial infarction model (Rat-MI)

MI was induced as previously described, with several modifications (Piao et al., 2005). Male SD rats were anesthetized with a mixture of ketamine hydrochloride (100 mg/kg body weight, i.m.) and xylazine (10 mg/kg body weight i.m.). After a tracheotomy had been performed, the rats' lungs were ventilated mechanically with positive pressure ventilation using a 30–40% air/oxygen mixture to maintain the arterial blood gas pH within a physiological range by adjusting the respiratory rate and tidal volume throughout the experiment. Myocardial infarction was induced by ligation of the left anterior descending artery (LAD). The thorax was opened at the fourth or fifth left intercostal space. After left thoracotomy and pericardiotomy, MI was induced by LAD ligation 2–3 mm from the origin with a 6-0 silk suture. Sham-operated rats served as surgical controls and were subjected to the same procedures as the experimental animals with the exception that the left anterior descending artery was not ligated. All animals (except for the rats in the sham-operated groups) were subjected to 40 min of regional myocardial