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MicroRNA-145 suppresses ROS-induced Ca^{2+} overload of cardiomyocytes by targeting CaMKIIδ

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ABSTRACT

A change in intracellular free calcium (Ca^{2+}) is a common signaling mechanism of reperfusion-induced cardiomyocyte death. Calcium/calmodulin dependent protein kinase II (CaMKII) is a critical regulator of Ca^{2+} signaling and mediates signaling pathways responsible for functions in the heart including hypertrophy, apoptosis, arrhythmia, and heart disease. MicroRNAs (miRNA) are involved in the regulation of cell response, including survival, proliferation, apoptosis, and development. However, the roles of miRNAs in Ca^{2+}-mediated apoptosis of cardiomyocytes are uncertain. Here, we determined the potential role of miRNA in the regulation of CaMKII dependent apoptosis and explored its underlying mechanism. To determine the potential roles of miRNAs in H_2O_2-mediated Ca^{2+} overload, we selected and tested 6 putative miRNAs that targeted CaMKIIδ, and showed that miR-145 represses CaMKIIδ protein expression and Ca^{2+} overload. We confirmed CaMKIIδ as a direct downstream target of miR-145. Furthermore, miR-145 regulates Ca^{2+}-related signals and ameliorates apoptosis. This study demonstrates that miR-145 regulates reactive oxygen species (ROS)-induced Ca^{2+} overload in cardiomyocytes. Thus, miR-145 affects ROS-mediated gene regulation and cellular injury responses.

1. Introduction

Myocardial infarction (MI) is associated with increased reactive oxygen species (ROS) production, heart failure, and increased mortality [1]. The calcium/calmodulin dependent protein kinase II (CaMKII) has emerged as a MI- and ROS-activated signaling molecule that regulates expression of apoptotic genes and affects adverse outcomes after MI [2–4]. Cardiac-specific transgenic overexpression of CaMKII results in cardiac hypertrophy, heart failure, and premature death [5]. CaMKII is constitutively activated by threonine-287 phosphorylation, and constitutively active CaMKII can lead to many of these MI-related adverse effects [6,7]. In recent *in vitro* experiments, we described an apoptotic pathway that involves increases in ROS produced by diesel exhaust particles, and activation of CaMKII [8].

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by targeting the 3′ untranslated region (3′UTR) of target mRNAs. As a result, translation is suppressed or target mRNA is rapidly degraded [9,10]. Recent studies showed that miRNAs are involved in cardiac physiology and pathology [11,12]. Many such studies have demonstrated that miRNAs can regulate cardiac apoptosis [13]. Myocardial-specific miR-1 and miR-133a may play an important role in cardiac apoptosis. miR-1 regulates cardiomyocyte apoptosis via post-transcriptional repression of IGF-1, Hsp60, and Bcl-2 [14–16]. A miR-133a mimic down-regulated caspase-9 protein expression and attenuated ischemia–reperfusion (I/R)-induced apoptosis [17]. miR-21, the miR-30 family, and miR-199a are anti-apoptotic miRNAs, and miR-195 and miR-320 are pro-apoptotic miRNAs [13,18].

A previous study demonstrated that miR-145 is abundantly expressed in smooth muscle [19]. One of the targets of miR-145 in vascular smooth muscle cells (VSMC) is CaMKII [20]. miR-145 in-

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duces smooth muscle cell (SMC) proliferation and differentiation [20,21]. Although miR-145 initiates apoptosis in cancer cells [22–24], the role of miR-145 in cardiomyocytes remains unclear. Therefore, our research focused on the effect of miR-145 expression in Ca^{2+} overload by activation of CaMKII in cardiomyocytes and determined whether miR-145 regulates ROS-induced cardiomyocyte apoptosis. Our data suggest that miR-145 may be a powerful therapeutic target for ischemic heart diseases.

2. Materials and methods

2.1. Isolation of rat ventricular cardiomyocytes

Animals were handled in compliance with the Guiding Principles in the Care and Use of Animals. All experimental procedures for animal studies were approved by the Committee for the Care and Use of Laboratory Animals, Yonsei University College of Medicine, and performed in accordance with the Committee's Guidelines and Regulations for Animal Care (NIH Publication No. 85-23, revised 1996). We isolated and purified neonatal rat cardiomyocytes. Briefly, 1- to 2-day-old Sprague Dawley rat pups were anesthetized with ether in batches of five at a time. Using microdissecting scissors, hearts were minced to pieces of approximately 1 mm^3 and the ventricles were washed with Dulbecco's phosphate-buffered saline solution (PBS, pH 7.4) free of Ca^{2+} and Mg^{2+}. The tissues were washed with PBS and enzymatically digested with 10 ml of collagenase II (0.8 mg/ml, 262 units/mg, Gibco BRL) for 5 min at 37 °C. The supernatant was then removed, and the tissue was treated with fresh collagenase II solution for an additional 5 min. The cells in the supernatant were transferred to a tube containing cell culture medium (α-MEM containing 10% fetal bovine serum (FBS), Gibco BRL). The tubes were centrifuged at 1200 rpm for 4 min at room temperature, and the cell pellets were resuspended in 5 ml of cell culture medium. The above procedures were repeated 7–9 times until little tissue remained. The cell suspensions were collected and incubated in 100 mm tissue culture dishes for 1–3 h to reduce fibroblast contamination. The non-adherent cells were collected and seeded to achieve a final concentration of 5×10^5 cells/ml. After incubation for 4–6 h, the cells were rinsed twice with cell culture medium, and 0.1 μM 5-bromo-2′-deoxyuridine (BrdU) was added in order to increase the purity of cardiomyocytes. The cells were then cultured with 10% (v/v) FBS in a CO_2 incubator at 37 °C.

2.2. Treatment of cells with hydrogen peroxide

One day following isolation, cardiomyocytes were rinsed twice with PBS. The cells were further incubated with α-MEM containing 1% FBS. Various concentrations of H_2O_2 were then added to the medium and cells were incubated for the indicated times. For negative controls, cells were incubated with medium lacking H_2O_2 for equivalent amounts of time.

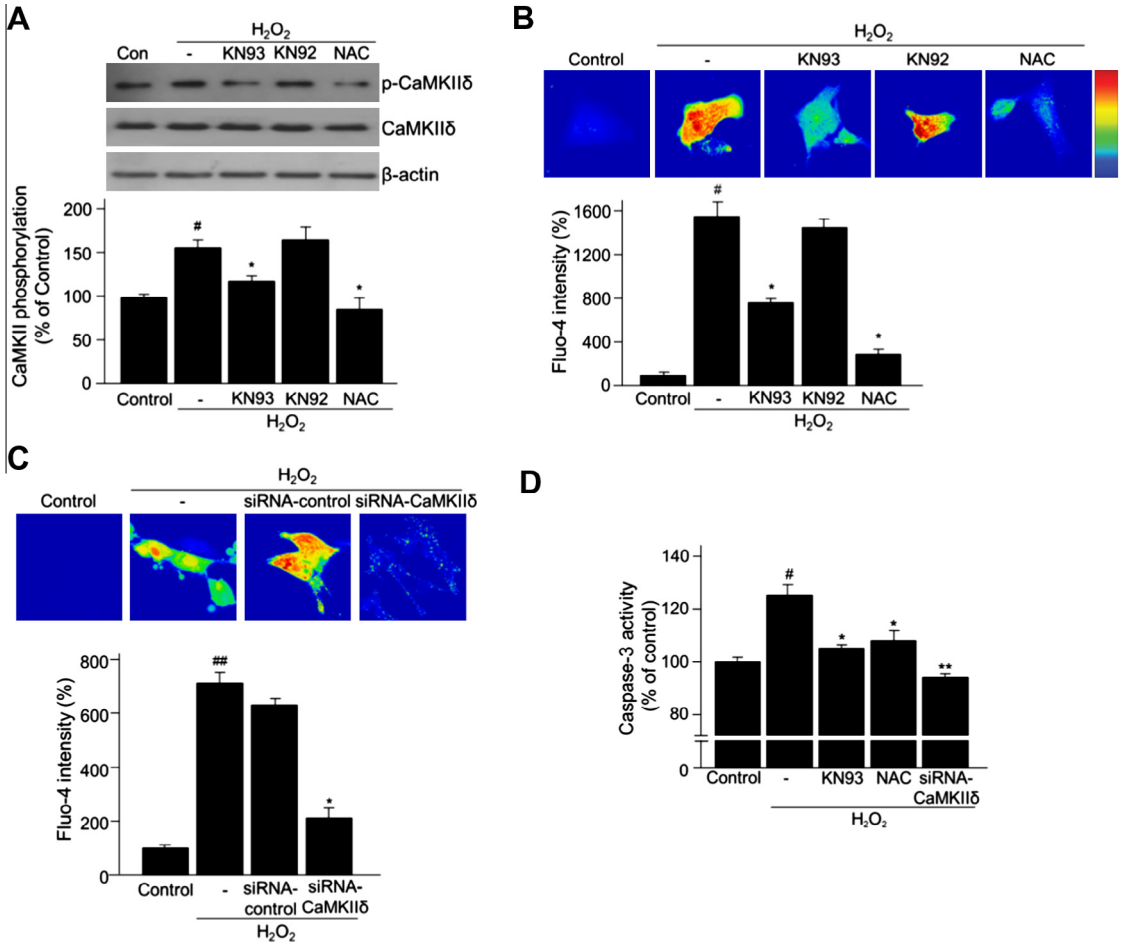

Fig. 1. H_2O_2 induces CaMKIIδ expression. (A) Western blot analysis of phosphorylated CaMKIIδ (p-CaMKIIδ), CaMKIIδ, and β-actin. (B) Cardiomyocytes treated with or without each inhibitor or analogue and H_2O_2. All inhibitors, including 5 μM KN93, 5 μM KN92, and 10 mM NAC were added for 30 min, then 100 nM H_2O_2 was added for 6 h. Cytosolic free Ca^{2+} concentration was determined by Fluo-4 intensity. (C) siRNA-CaMKIIδ was added for 6 h in a dose-dependent manner. (D) Apoptosis was measured by caspase-3 activity assay ($^{\#}p < 0.05$, $^{\#\#}p < 0.001$ vs control, $^{*}p < 0.05$, $^{**}p < 0.001$ vs H_2O_2).