

# Cardiomyocyte Ischemia Model microRNA-210 Plays a Cytoprotective Role by Inhibiting BNIP3, Apoptosis and Autophagy Signal Pathway

FENLI ZHANG AND YONGCHAO ZHANG<sup>1\*</sup>

Department of Cardiology, First Affiliated Hospital of Xi'an Jiaotong University, Shaanxi Province 710061, 1 Electrocardiogram Department, Xi'an Fifth Hospital, Xi'an, Shaanxi Province 710082, China

Zhang *et al.*: To Explore the Protective Mechanism of Cardiomyocyte Ischemia Model

To explore the protective mechanism of cardiomyocyte ischemia model microRNA-210 by inhibiting adenovirus EIB interacting protein 3 and inhibiting apoptosis and autophagy signal pathway. Rat H9c2 cells were collected and cultured. Rat H9c2 cells were randomly divided into blank control (group A), model (group B), microRNA-210 (group C) and microRNA-210 inhibition group (group D). Group B, C and D were cultured in hypoxia to establish myocardial ischemia model. After successful modeling, the group B did not do any treatment. Lentivirus transfection was used in group C and D to construct cells with high expression of microRNA-210 and low expression of microRNA-210 inhibition. Western blotting was used to check the adenovirus EIB interacting protein 3, autophagy-related index, apoptosis-related index protein and LC3BI/LC3B2II ratio in H9c2 cells of rats in each group. The microRNA-210 in the group B was reduced than the group A, while the adenovirus EIB interacting protein 3 was raised than the group A. The microRNA-210 in the group C was raised than the group B, while the adenovirus EIB interacting protein 3 was decreased than the group B. There was no significant difference in microRNA-210 and adenovirus EIB interacting protein 3 in group D. The microRNA-210 decreases and adenovirus EIB interacting protein 3 increases in the model of cardiomyocyte ischemia. MicroRNA-210 plays a protective role in the process of cardiomyocyte ischemia, which may be achieved by inhibiting adenovirus EIB interacting protein 3 and hindering apoptosis and autophagy signal pathway.

**Key words:** Myocardial ischemia, microRNA-210, adenovirus EIB interacting protein 3, apoptosis, autophagy signal

Cardiovascular Disease (CVD) is the main cause of human death worldwide, and ischemic injury is an important factor leading to CVD<sup>[1]</sup>. It has been reported that timely reperfusion is beneficial to promote the recovery of hypoxic tissue, but the longer the hypoxia time is, the more serious the injured tissue injury is, which is called ischemia-reperfusion injury<sup>[2]</sup>. Ischemia-reperfusion injury is closely related to physiological and pathological processes such as apoptosis, autophagy and necrosis, in which autophagy has a certain regulatory function in CVD<sup>[3]</sup>. Therefore, exploring the possible regulatory factors of autophagy has been the focus in the field of cytology. microRNA-210 (miR-210), a member of the miRNAs family, has become a recognized hypoxia-

related miRNA and can play a stable biological effect in many cell lines<sup>[4]</sup>. MiR-210 can control many genes, but only some genes can confirm its regulatory function in CVD<sup>[5]</sup>. Adenovirus EIB Interacting Protein 3 (BNIP3) is a hypoxia-regulated protein with diverse functions in the process of apoptosis<sup>[6]</sup>. Clinical studies have shown that miR-210 can participate in a variety of cellular processes by regulating B-Cell Lymphoma 2 (Bcl-2)/BNIP3, which can affect cell autophagy. Other

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\*Address for correspondence

E-mail: wangkatg345482@163.com

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studies have confirmed that BNIP3 can be highly expressed in CVD, and blocking the expression of this gene can alleviate the degree of myocardial damage, but its significance in the diagnosis and treatment of CVD is not clear<sup>[7,8]</sup>. Therefore, in this study, the rat cardiomyocyte H9c2 was used to establish the model of myocardial ischemia, and this study aimed to explore the mechanism of miR-210's cytoprotective effect by inhibiting BNIP3 and then blocking apoptosis and autophagy signal pathway. Rat H9c2 cells were purchased from Wuxi Xinrun Biotechnology Co., Ltd., and all cells were incubated in high-glucose Dulbecco's Modified Eagle Medium (DMEM) (containing 10 % Fetal Bovine Serum (FBS)) and incubated in Carbon dioxide (CO<sub>2</sub>) incubator (37°). After a series of operations such as resuscitation, passage and cryopreservation, they were cryopreserved. Rat H9c2 cells were randomly divided into blank control (group A), model (group B), miR-210 (group C) and miR-210 inhibition group (group D). Group B, C and D were cultured in hypoxia to establish myocardial ischemia model. After successful modeling, the group B did not do any treatment. Lentivirus transfection was used in group C and D to construct cells with high expression of miR-210 and low expression of miR-210 inhibition. Western blotting was used to detect miR-210, BNIP3, autophagy related indexes (microtubule associated protein 1 Light Chain 3 (LC3B1), LC3B2), apoptosis related indexes (caspase-3), Bcl-2, Bcl-2-Associated protein X (BAX)) protein expression and LC3BI/LC3B2II ratio in H9c2 cells of rats in group A, B, C and D. The miR-210 and BNIP3 in each group were expressed by ( $\bar{x} \pm s$ ). T-test was used for comparison between the two groups, and multivariate analysis of variance was used for comparison between groups. All data in this study were analyzed by Statistical Package for the Social Sciences (SPSS) 19.0.  $p < 0.05$  was considered to be statistically significant and compared with the group A, <sup>a</sup> $p < 0.05$  and compared with the group B, <sup>b</sup> $p < 0.05$ . The miR-210 in the group B was reduced than the group A, while the BNIP3 protein in the group B was raised than the group A. The miR-210 in the group C was raised than the group B, while the BNIP3 protein was decreased than the group B (Table 1 and fig. 1). The LC3B1 and LC3B2 protein and the ratio of LC3BI/LC3B2II in the group B were raised than the group A, while these in group C were reduced

than the group B, and these in group D were increased than the group B (Table 2 and fig. 2). The caspase-3 and BAX protein in the group B was raised than the group A, while the Bcl-2 protein was reduced than the group A. The protein caspase-3 and Bax in the group C was decreased than the group B, while the Bcl-2 protein was increased than the group B (Table 3 and fig. 3). MiR-210 has become the focus of current researchers, and the research on it is very extensive. Clinical studies have shown that Hypoxia-Inducible Factor 1-Alpha (HIF-1 $\alpha$ ) can down-regulate the miR-210 in knockout mice. Through co-immunoprecipitation reaction, other scholars found that HIF-1 $\alpha$  usually binds to the miR-210 promoter under hypoxia compared with cells with normal oxygen content<sup>[9]</sup>. Animal experiments have confirmed that some drugs, such as Huoxue Anxin recipe, can induce the up-regulation of miR-210 expression<sup>[10]</sup>. In addition, serum reactive factor can stimulate many miRNAs activation, which is an exogenous activator. MiR-210 can regulate the hypoxia response of cardiovascular system through apoptosis, autophagy, migration and other biological aspects, and play its protective role. The most common role of this gene is to block apoptosis and alleviate cell survival. A number of reports have shown that the increase of its expression level can significantly reduce the rate of apoptosis<sup>[11]</sup>. Other scholars have found that in hydrogen peroxide moral mouse cardiomyocyte model, miR-210 can block the expression of apoptosis-promoting gene caspase-8, and finally inhibit apoptosis<sup>[12]</sup>. Cells stimulated by hypoxia are mainly apoptotic, and the up-regulation of BNIP3 expression can promote apoptosis<sup>[13]</sup>. Some scholars found that hypertrophic HL-1 cardiomyocytes were used to establish hypoxia model, and found that BNIP3 accumulated obviously. In addition, it can be used as a key protein of hypoxic cell necrosis and apoptosis. Other studies found that mutants with Bcl-2 gene mutations could not bind to BNIP3, accompanied by the decrease of anti-apoptotic effect of Bcl-2. It is suggested that BNIP3 can bind to anti-apoptotic proteins to play a protective role<sup>[14]</sup>. Autophagy is a process involving the degradation of longevity proteins and organelles. Some scholars have established a malignant glioma model by ceramide stimulation. The results show that BNIP3 can play an autophagy control function in autophagy

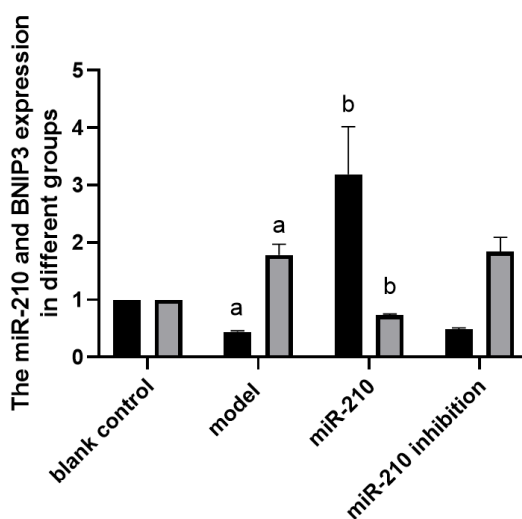
death<sup>[15]</sup>. Another study found that BNIP3 can induce autophagy and protect hypertrophic HL-1 cardiomyocytes from ischemia-reperfusion injury<sup>[16]</sup>. Here, the ischemia model of cardiomyocytes was established by anoxic culture, and the high expression of miR-210 and low expression of miR-210 inhibition were constructed by lentivirus transfection, and the negative control group and group A were set up to explore whether miR-210 can protect cardiomyocytes by inhibiting BNIP3 from apoptosis and autophagy. First of all, we detected the expression of miR-210 and BNIP3 protein in the myocardial ischemia model. The miR-210 in the group B was reduced than the group A, while the BNIP3 protein in the group B was raised than the group A. The miR-210 in the group C was increased than the group B, while the BNIP3 protein was reduced than the group B. It is suggested that miR-210 and BNIP3 are involved in the whole process of cardiomyocyte ischemia, and the expression of miR-210 is low and BNIP3 is high in this disease. In addition, we further detected the expression level of autophagy-related proteins in each group. The LC3B1 and LC3B2 protein and the ratio of LC3BI/LC3B2II in the

group B were raised than the group A. The LC3B1 and LC3B2 protein and the ratio of LC3BI/LC3B2II in group C were reduced than the group B, while these in group D were increased than the group B. It is suggested that up-regulation of miR-210 can block the LC3BI and LC3B2II, while down-regulation of miR-210 can prick them. Finally, we detected whether miR-210 can participate in the regulation of BNIP3 and then control apoptosis. The results showed that the caspase-3 and BAX protein in the group B was increased than the group A, while the Bcl-2 protein was decreased than the group A. The caspase-3 and Bax in the group C was reduced than the group B, while the Bcl-2 protein was raised than the group B. It is suggested that up-regulating of miR-210 can inhibit the BNIP3 and further inhibit apoptosis, which has the function of cell protection. To sum up, the expression level of miR-210 decreased and the expression of BNIP3 increased in the model of cardiomyocyte ischemia. MiR-210 plays a protective role in the process of cardiomyocyte ischemia, which may be achieved by inhibiting BNIP3 and blocking apoptosis and autophagy signal pathway.

**TABLE 1: COMPARISON OF miR-210 AND BNIP3 IN EXPRESSION IN DIFFERENT GROUPS ( $\bar{x}\pm s$ )**

Group	miR-210	BNIP3 protein
A	1.00	1.00
B	0.44±0.02 <sup>a</sup>	1.78±0.19 <sup>a</sup>
C	3.19±0.83 <sup>b</sup>	0.73±0.02 <sup>b</sup>
D	0.48±0.03	1.84±0.25

Note: Compared with the group B, <sup>a</sup>p<0.05 and compared with the group C, <sup>b</sup>p<0.05



**Fig. 1: The miR-210 and BNIP3 expression in different groups**

Note: (■): miR-210 and (▒): BNIP3

**TABLE 2: COMPARISON OF EXPRESSION LEVELS OF AUTOPHAGY-RELATED PROTEINS ( $\bar{x}\pm s$ )**

Group	LC3BI/LC3BII
A	1.00
B	2.00±0.01
C	0.66±0.04 <sup>a</sup>
D	2.26±0.47 <sup>ab</sup>

Note: Compared with the group C, <sup>a</sup>p<0.05 and compared with the group D, <sup>b</sup>p<0.05

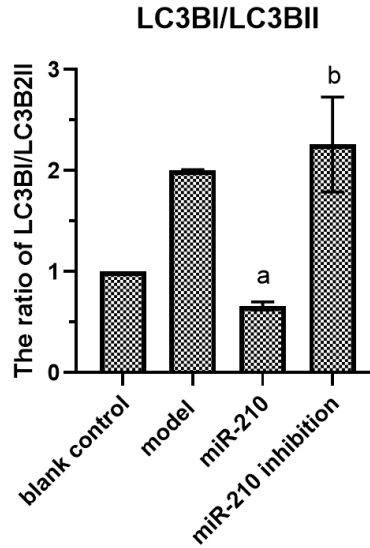


Fig. 2: Comparison of expression levels of autophagy-related proteins

**TABLE 3: COMPARISON OF EXPRESSION LEVELS OF APOPTOSIS-RELATED PROTEINS ( $\bar{x}\pm s$ )**

Group	Caspase-3	Bcl-2	BAX
A	1.05±0.07	1.02±0.05	1.04±0.08
B	2.51±0.15	0.59±0.02	2.67±0.31
C	0.54±0.09 <sup>a</sup>	1.23±0.06 <sup>ba</sup>	0.49±0.04 <sup>a</sup>
D	2.86±0.23 <sup>b</sup>	0.51±0.01 <sup>b</sup>	2.88±0.38 <sup>b</sup>

Note: Compared with the group C, <sup>a</sup>p<0.05 and compared with the group D, <sup>b</sup>p<0.05

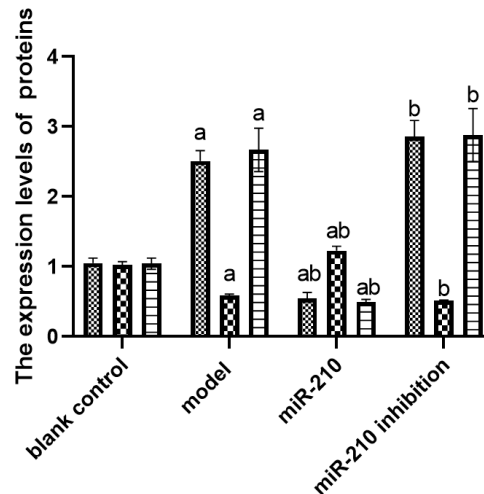


Fig. 3: Comparison of expression levels of apoptosis-related proteins

Note: (▨): Caspase-3; (▩): Bcl-2 and (▤): BAX

**Conflict of interests:**

The authors declared no conflict of interests.

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