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The effect of MiR-497 on the expression of target genes BCL-2 and LC3B on cardiomyocytes injured by hypoxia/reoxygenation

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ABSTRACT

Myocardial ischemia is easy to cause hypoxia or necrosis of myocardial cells. At present, the performance of various patients is different. Basically, it is mainly caused by chest pain or chest discomfort. Severe patients may die suddenly. Therefore, looking for effective drugs or methods to prevent and treat Cardiomyocyte injury is of great significance for clinical practice, in which the expression of regulatory gene BCL-2 and microtubule-associated protein light chain 3B (LC3B) has a certain effect on hypoxia/reoxygenation injured cardiomyocytes. To this end, the team designed a study on the effect of miR-497 on the expression of target genes BCL-2 and LC3B on cardiomyocytes injured by hypoxia/reoxygenation. In this study, a control group experiment was set up for the study. During the experiment, the cells were treated with hypoxia-reoxygenation and transfected with the corresponding miR-497 treated cells. By detecting apoptosis, the kit was used to detect cell activity and RT-PCR detection. Gene expression levels and other methods are comparatively judged. The results of this study showed that compared with the normal group 14.50±0.78, the viability of cardiomyocytes in the model group was significantly reduced (P<0.01), the amount of NO released by cardiomyocytes was reduced (P<0.01), and the protein expression in cardiomyocytes was significantly reduced (P<0.01). The experimental results of this study prove that miR-497 can alleviate the damage caused by hypoxia-reoxygenation of cardiomyocytes by regulating target genes BCL-2 and LC3B.

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Introduction

Ischemic heart disease is common clinical heart disease. When no blood passes through the heart, it will cause an imbalance of oxygen supply, resulting in damage to the myocardial tissue and dysfunction. However, severe damage caused by hypoxia/reoxygenation of cardiomyocytes is one of the main reasons for the poor prognosis of surgical treatment of this disease. miR-497 has played an important role in the development and treatment of ischemic heart disease. In recent years, there have been many studies on miRNA treatment of heart disease. Therefore, studying the effect of miR-497 on hypoxia/reoxygenation-damaged cardiomyocytes has become a very promising new idea. Some studies have shown that miR-497 can start by regulating the target genes BCL-2 and LC3B. In order to alleviate the damage caused by hypoxia-reoxygenation of cardiomyocytes, it is of important reference value to study the effect of miR-497 on the expression of target genes BCL-2 and LC3B on cardiomyocytes injured by hypoxia/reoxygenation (1-3).

According to the severity of the disease, myocardial cell injury can be divided into myocardial ischemia, myocardial injury, myocardial necrosis and so on. In contrast, due to the different formation mechanisms of various cardiomyocyte models, the cardiomyocyte injury model plays a more and more important role in related drug mechanisms, new drug research and development, and cardiotoxicity research. Huang Y suggested that age-related oxidative DNA damage was not associated with decreased proliferation of human cardiomyocytes (1). He analyzed 60 samples of ventricular septal defect (VSD) myocardial tissue taken from infants during congenital heart operation (2-3). His research results show that oxidative DNA damage cannot fully explain the reasons for the decrease in human cardiomyocyte proliferation (4-5). The results of this study open up a way for the study of myocardial cell injury (6-7). In the following study, Huang Y further studied the effect of microscale

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corrosion on myocardial production and secretion by using polyetherimide cell culture inserts with different bottom roughness (8). The results of this study provide a certain reference for the study of cardiomyocytes, and we need to further prove this (9-10). At present, there are many researches on target gene expression, and many scholars have studied gene transfection technology for target gene expression. For example, Soldner F's team studies genome-wide association, which regulates GWAS in DNA elements by establishing an experimental system for precise gene control(11). His study confirmed that the transcriptional release of SNCA is related to the sequence-dependent binding of brain-specific transcription factors Emx2 and nkx6-1 (12-13). This experiment is very representative, linking genetic variation with disease-related phenotype function (14-15). In the study, Soldner Fused five common RNA extraction methods on the market to extract RNA from mouse lung, liver and brain tissues, which verified that different methods of miRNA separation would produce different results (16-17). His research aims to optimize these methods for the specific application and has certain innovations (18-19).

The aim of this study is to investigate the effect of miR-497 on the hypoxia/reoxygenation injury of cardiomyocytes by regulating the expression of BCL-2 and LC3B. In order to solve the problem of detecting apoptosis-related proteins in cardiomyocytes, a control group experiment was set up to study.

During the experiment, the cells were treated with hypoxia-reoxygenation and transfected with the corresponding mir-497 treatment cells. The results showed that 14.50 ± 0.78 in the control group were compared with that in the normal group by detecting apoptosis, detecting cell activity with the kit and detecting gene expression level with RT-PCR Compared with the control group, the activity of myocardial cells decreased significantly ($P < 0.01$), and the no release of myocardial cells decreased ($P < 0.01$). MiR-497 can target BCL-2 and LC3B, and alleviate the injury caused by anoxia and reoxygenation of myocardial cells. The innovation of this paper is that the selected data is detailed and the academic research on miR-497 is not very thorough.

Cardiomyocyte Damage

Ischemic cardiomyopathy has a high incidence in the clinic. Myocardial ischemia is easy to cause hypoxia or necrosis of myocardial cells. At present, the performance of various patients is different, mainly causing chest pain or discomfort. Severe patients may die suddenly, which has become a problem that has to be paid attention to. Generally speaking, the heart muscle refers to the heart muscle cells, when the heart muscle cells appear ischemia, inflammation, hypertrophy, necrosis and other pathological conditions, there will be myocardial damage. But usually what our doctors call myocardial injury is caused by myocarditis. Myocardial necrosis is caused by myocardial infarction of coronary heart disease, which is different (20-21).

Some mRNA can inhibit the apoptosis of anoxia reoxygenation cardiomyocytes and play a protective role. Its mechanism is related to the targeted regulation of HMGB1 expression, which will lay a foundation for the treatment of cardiovascular diseases and provide a new target (22).

LC3B

The protein encoded by the microtubule-associated protein 1 light chain 3 gene is the first autophagy marker protein, which can be used as a representative protein to study autophagy. LC3 gene is of great value to the formation of autophagy. Autophagy is a process in which cells use lysosomes to degrade damaged organelles and proteins and maintain a stable intracellular environment. It is not only an important regulatory mechanism for cell growth and development, differentiation, maturation and death, but also closely related to the occurrence and development of tumors.

LC3 has been used as a marker of autophagy to detect autophagy activity. Apoptosis, also known as type I programmed cell death, is regulated by a variety of apoptosis-related genes (such as p53, BCL-2, etc.). Autophagy and apoptotic genes are closely related to the differentiation degree of tumor cells and the clinicopathological stages of tumors (23).

MiR-497

MicroRNA, also known as microRNA, microRNAs (microRNAs, MiRs) are a kind of non-coding microRNA found in eukaryotes in recent years, which

regulate genes after transcription. They can make the target gene mRNA degradation or translation blocked, and then play a similar role in promoting or inhibiting cancer genes. Mir-497 is located on human chromosome 17, which is a hot research topic (24). MicroRNAs are highly conserved noncoding microRNAs that regulate the expression of target genes at the post-transcriptional level. Recently, it has been found that microRNA, as an endogenous non-coding microRNA, is stable in the body. It is involved in the pathogenesis of many diseases by regulating the expression of its target genes. Differential expression of microRNA in the body may be closely related to cancer, diabetes, cardiovascular disease and lung disease (25).

Materials and methods

Experimental Setup

In this study, a control experiment was used to improve the accuracy of the results of this study. In this study, the cardiomyocytes were divided into three groups. During the experiment, the cells were treated with hypoxia-reoxygenation and transfected with miR-497. The apoptosis was detected, the cell activity was detected by kit and the gene expression was detected by RT-PCR.

Cell Culture and Treatment

The cells were cultured in a DMEM medium containing 10% fetal bovine serum, 2 mM glutamine and 1% penicillin. The cells were cultured at 5% CO2 and 36 ℃ for 1 day and then changed for 2-3 days. The cells were divided into the control group, the hypoxia-reoxygenation (H / R) group and the mir-497 group. In addition to the CTRL group, the other groups were treated with Tyrode solution at 95% N2, 5% CO2 and 36 ℃, resulting in hypoxia injury. After that, they were cultured in 5% CO2 and 36 ℃ for 6h, 12h and 24h respectively, RT-PCR was used to detect gene expression levels. After reoxygenation for 24h, they were used for subsequent experiments.

Western Blotting

Take the appropriate amount of 1.2.2 cells of each group in the logarithmic growth period, split them with Ripa, extract the total protein, denature the protein quantitatively by BCA method, carry out protein electrophoresis, then carry out PVDF membrane transfer, seal them for 2h, and then incubate them with anti-I at 4 ℃ overnight. The next day, the membrane was washed and incubated at 36 ℃ for 2 hours. After that, add the development mixture and expose the development. The expression of HMGB1, B-cell lymphoma / leukemia-2 (BCL-2), Bax and caspase-3 was expressed by the ratio of the gray value of the target band to the gray value of β - actin.

Experimental Operation

Place the experimental equipment and consumables on the ultra-clean table wiped with alcohol, and irradiate the ultraviolet light for more than 30min. Prepare a concentration of 0.1% pancreatin and 0.08% type II collagenase with HBSS diluted 10 times and filter for use. Take the suckling rats, moisten and disinfect in 75% alcohol, cut the chest cavity along the left side of the sternum xiphoid to expose the heart, the ophthalmic forceps clamp the heart against the apex of the heart, bluntly separate the heart, put the heart into the pre-cooling wash the HBSS Petri dish 3 times to remove blood from the heart as much as possible. The pericardium, large blood vessels and auricle tissue removed from the heart were transferred to a 10mL serum bottle, and the myocardial tissue was cut into lmm3 tissue blocks with ophthalmic scissors. Add 5mL of 0.1% pancreatin and 0.08% type II collagenase mixed digestion solution, gently pipette to mix, digest with magnetic stirring (200rpm/min) at 36°C for 5min, discard the first digested supernatant, and add the mixture The digestion solution continues to be digested, and the supernatant is collected and added to 5 mL of pre-chilled DMEM medium containing 10% fetal bovine serum for neutralization, mixed upside down and placed on ice. Repeat the above digestion process about 12 times until myocardial tissue is basically digested. The cell suspension was centrifuged in DMEM medium placed on ice, the supernatant was removed, resuspended with 10 mL of 10% fetal bovine serum DMEM medium containing BrdU, and filtered on a 100 mesh filter. The filtered cell suspension was spread into a 10cm Petri dish, placed in a 5% CO2 and 36°C incubator and allowed to stand for 1.5h, and the fibroblasts were removed with differential adhesion. After the differential adhesion, the non-adherent

myocardial cell suspension is sucked out, suspended in DMEM culture medium containing 10% fetal bovine serum of BrdU, the cell count is performed with a cell counting plate, and the cells are seeded at a density of 5×105/ml Culture plate. Put 5% CO2 and cells in a 36°C cell incubator for 48h for normal culture (change the medium every 2 days).

Statistical Analysis

The experimental data was analyzed using SPSS22.0 software. In order to ensure the accuracy of the experimental results, the cell experiment results were repeated at least 3 times. The experimental data were expressed by (x±s). The comparison between the two groups was by t test, with P<0.05. The difference is statistically significant.

Results

MiR-497 Regulates Target Gene Results

miR-497 regulates the target gene through a specific pathway. MiR-497 is located on human chromosome 17. Through a specific pathway, it has an impact on cardiac myocytes and detects the appropriate amount of logarithmic cell fluorescence intensity, the results are shown in Figure 1. It can be seen from Figure 1 that the trend of the two groups is obviously different. With the time of 6 hours, 12 hours and 24 hours, the injured cardiomyocytes in the experimental group gradually recover from 1.05 in 6 hours to 1.6 in 24 hours. Compared with the miR-497 group, luciferase activity of the control group without any treatment decreased significantly (P < 0.01), and the difference was statistically significant.

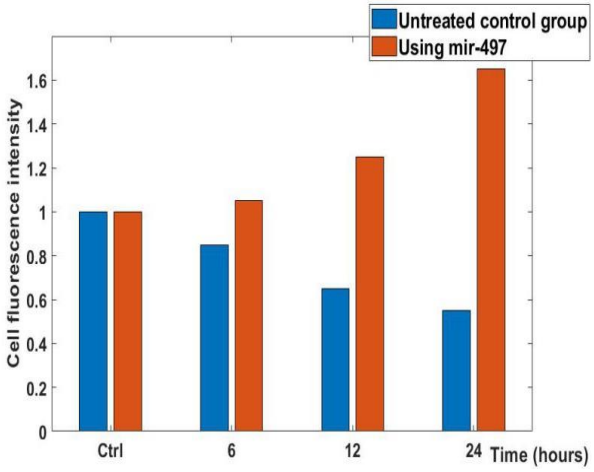

Figure 1. Fluorescence intensity of cells in experimental group and control group

Relative Expression of Target Genes BCL-2 and LC3B

Western blot analysis of the relative expression levels of BCL-2 and LC3B affected by miR-497 is shown in Table 1.

Table 1. Expression of two histones(x±s)

Group	8h	24h	48h	72h
BCL-2	1.53±0.46	2.06±0.10	2.71±0.45	3.58±0.32
LC3B	1.50±0.39	2.31±0.41	2.61±0.56	3.01±0.46
t	0.701	2.765	4.331	6.147
P	0.522	0.048	0.007	0.001

It can be seen from Table 1 that the relative expression levels of the two groups of proteins increased after transfection by Western blot analysis, but the upward trends were different. Among them, the BCL-2 group began to rise gradually from 1.53 to 2.06 and finally to the level of 3.5, LC3B first quickly increased from 1.5 to 2.31, and finally gradually slowed down to 3.01. The t values are all normal, 0.701 for 8 hours, 2.765 for 24 hours, 4.331 for 48 hours, and 6.147 for the last 72 hours. Protein expression detection the ratio of the absorbance values of the two groups of cells at different times is shown in Figure 2.

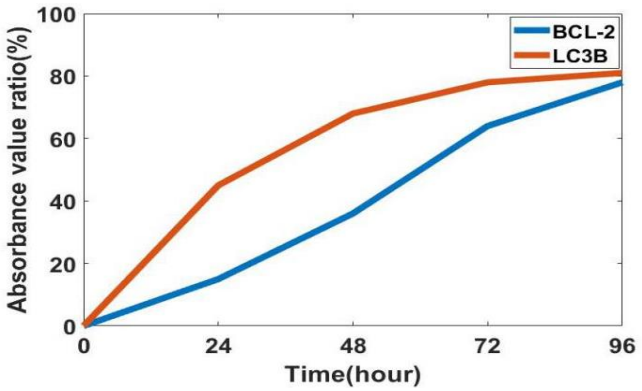

Figure 2. Protein expression detection Ratio of absorbance values of two groups of cells at different times

It can be seen from Figure 2 that the expressions of the two target genes BCL-2 and LC3B are both increasing, which is consistent with the conclusion of Table 1. We can see that the ratio of the absorbance of the target gene BCL-2 expression increases from about 20 in 24h to 96h. 76, and LC3B expression reached about 48 at the beginning, and then slowly reached 81 in 96h. Compared with the control group,

BCL-2 (P<0.05), and LC3B (P<0.05), the difference was statistically significant.

RT-PCR to Detect miR-497 Expression

The miR-497 expression cell line was constructed, and after RT-PCR detection, the relative expression of miR-497 was detected by RT-PCR in three groups of cardiomyocytes as shown in Figure 3.

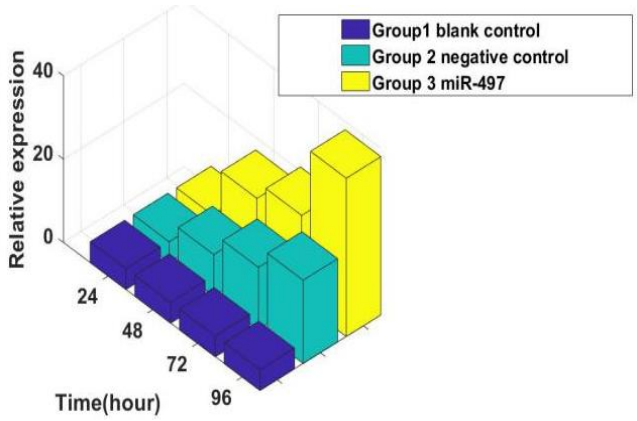

Figure 3. Detection of relative expression of mir-497 by RT-PCR in cardiac myocytes

As can be seen from Figure 3, the relative expression levels of miR-497 in the three groups of cardiomyocytes detected by RT-PCR were significantly different. The blank control was always the initial value of 5 because it suppressed expression by means of control, and served as the control. The control group rose slowly from 5 to 20, and the miR-497 group showed a stepwise increase. The test results showed that the relative expression level of miR-497 was significantly higher than that of the blank control group and the negative control group (P<0.01). There was no statistically significant difference in the expression level between the blank control group and the negative control group (P>0.05).

In order to further prove the effect of damaged cardiomyocytes, the lateral comparison with other means, by comparing the MDA and T-SOD levels of cardiomyocytes, as shown in Figure 4.

As can be seen from Figure 4, compared with the Ctrl group, the apoptosis rate of cardiomyocytes in the H/R group increased significantly (P<0.01), and the difference was statistically significant; compared with the H/R group, the apoptosis rate of the cardiomyocytes in the miR-497 group Significantly reduced (P<0.01), the difference was statistically significant. By comparing heart surgery in infants and young children, the study showed that IL-6 showed a significant increase at all-time points after surgery, which was confirmed by experiments. The control group T-SOD is always maintained at 5, because no treatment is performed; in the hypoxia/reoxygenation group, that is, the H/R performance is very high, and miR-497 is relatively stable, which is consistent with the results of miR-497 by regulating the target gene meets the.

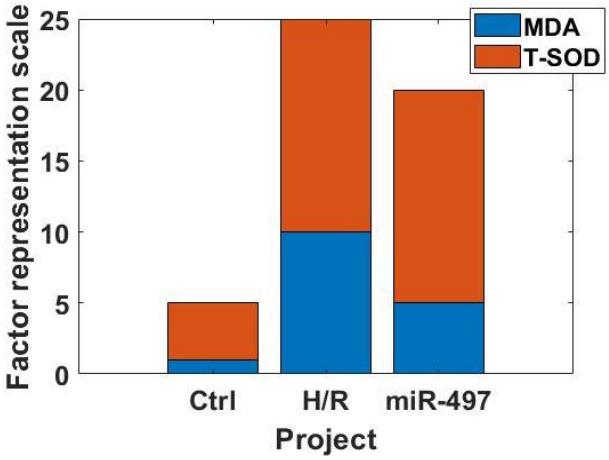

Figure 4. MDA and T-SOD levels in cardiomyocytes

Discussion

Myocardial ischemia is easy to cause hypoxia or necrosis of myocardial cells. The performance of various patients is currently different. Basically, it is mainly caused by chest pain or chest discomfort. Severe patients may have sudden death. Research on the effects of oxygen damage on cardiomyocytes is still relatively rare in academia. Therefore, it is particularly important to study miR-497 in the development of ischemic heart disease, and studies have shown that miR-497 regulates the expression of target genes BCL-2 and LC3B on myocardial cells injured by hypoxia/reoxygenation. The study observed the effects of hypoxia/reoxygenation on injured cardiomyocytes and explored the expression of miR-497 by regulating the expression of target genes BCL-2 and LC3B.

In this paper, a control group was set up for the experiment. The blank control group and the control group were set to ensure the accuracy of the experiment in this study. During the experiment, the

cells were treated with hypoxia and reoxygenation, and the corresponding miR-497 treated cells were transfected. Apoptosis, kit to detect cell activity and RT-PCR to detect gene expression levels are comparative judgments. The results of this study show that the experimental results of this study prove that miR-497 can alleviate the damage caused by hypoxia-reoxygenation of cardiomyocytes by regulating target genes BCL-2 and LC3B.

In addition, in order to further prove the effect of damaged cardiomyocytes, this study also compared with other methods laterally, by comparing the MDA and T-SOD levels of cardiomyocytes with cell irradiation experiments, and further verified the above conclusions. It will provide a reference value for clinical treatment. Due to the limited cost and the limitation of important experimental materials, this study still has some shortcomings. In the later period, the role and mechanism of miR-497 on other aspects of cardiomyocyte hypoxia and reoxygenation will be further studied and discussed.

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

Author's contribution

All authors responsible for the manuscript equally.

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