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MiR155 Relieves Acute Heart Transplantation in Mice by Modulating Th1/Th17 Immune Response

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ABSTRACT

Heart transplantation is an effective method for the treatment of end-stage heart disease. Therefore, this article aimed to establish a stable and effective mouse abdominal heart transplantation model. MiR155 alleviates the acute heart transplantation response by regulating Th1 / Th17 immune cytokines. This paper used the control method of randomly selecting samples to classify 30 healthy mice that met the conditions. First, C57BL / 6 mice were used as recipients, and Balb / c mouse hearts were used as donors to establish mouse hearts as a transplantation acute reaction model. A chronic rejection model of mouse heart transplantation was established by C57BL / 6 mice as recipients and Bm12 mouse hearts as donors. The survival time of the two groups of transplanted hearts was carefully recorded. The results of the study showed that in the heart transplantation acute/chronic rejection model, the average survival time of the donor's heart in the allograft group was (7.5 ± 0.37) / (63.4 ± 4.37) days, which was the same compared with the two groups. Therefore, in-depth analysis of the experimental control results and conclusions from the experimental results of the mice, this study can better respond to the pathological changes of acute/chronic rejection and reach the standard of model establishment. MiR155 relieves heart transplantation by regulating Th1 / Th17 immune response acute reaction.

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Introduction

In recent years, clinical medicine has gradually discovered that miR155, as a new immune-regulatory factor, plays an important role in the growth and development of immune cells, proliferation and differentiation, and the monitoring and regulation of the body's immune response (1). At the same time, with the progress of Th1 / Th17 cytokine research, it was found that under normal circumstances, the body's Th1 / Th17 function is in a dynamic balance, maintaining normal cellular immunity and humoral immune function disease (2). Heart transplantation is mainly a surgical transplant operation for advanced congestive heart failure and severe coronary artery disease. It is an allograft surgery in which the human heart that has been determined to be brain dead and successfully matched is completely removed and implanted in the chest cavity of the desired recipient. Heart transplantation is a high-risk operation, and the mortality rate in the hospital is about 7% (3). Postoperative cardiac complications include infection, sepsis, donor heart failure, bleeding, coronary atherosclerosis, chronic renal failure, immune rejection, and side effects of taking immunosuppressants (4). Because heart transplantation belongs to allogeneic organ transplantation, the recipient has the possibility of immune rejection (5). Some patients may have renal insufficiency after the operation (6). Therefore, it is important to focus on the relationship between miR155 and T cells in the immune system. Thl7 cells are mainly responsible for cellular immune responses, stimulating delayed-type hypersensitivity, enhancing cytotoxicity of killer cells, and assisting B cells to produce antibodies related to phagocytosis (2). Th17 is a helper T cell differentiated by TH0 cells under the stimulation of IL6 and IL23 (7). It mainly secretes pro-inflammatory factors such as IL17 and IL22. RORγ is an important transcription factor. TH17 cells play an important role in autoimmunity (8).

Based on recent research, the field of transplantation analyzes the miRNAs of the graft and

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the peripheral blood. We found that in almost all organ transplantation studies, miR155 expression will increase (9). In the rapid rejection of many organ transplants, miR155 expression was also confirmed to be significantly increased. miR155 plays an important role in regulating the differentiation of stem cell lines and the function of CD4 + T cells (10). MicroRNA is a kind of highly conserved short-chain non-coding RNA, which is stable in various body fluids of the human body (11). It participates in cell growth, differentiation, metabolism, and apoptosis—multiple biological processes (12). As one of the important members of miRNAs, miR-155 regulates various cell functions, such as hematopoiesis, immune response, viral infection, inflammatory response, etc. (13). It participates in the formation of diseases by regulating targeted mRNA and mediating the expression of various cytokines (14). This article further elaborated the research progress of the relationship between miR-155 and heart transplantation, hoping that miR-155 could provide a new direction for diagnosing and treating heart transplantation (15).

This article cuts in from the perspective of heart transplantation and miR155 and solves the problem of the role and mechanism of miR-155 in heart transplant rejection. Therefore, the method of constructing heart transplantation models of acute rejection and chronic rejection separately was used to explore the genetically engineered mice knocked out by miR-155 to study the role and molecular mechanism of miR-155 in heart transplantation. The innovations of this paper include the use of Balb / c and Bm12 mouse hearts as donors and C57BL / 6 as recipients to establish models of acute rejection and chronic rejection of heart transplantation, respectively, enriching the regulation of miR-1 SS The cognition of the mechanism provides new ideas and directions for the treatment of transplant rejection and provides a research basis for subsequent research.

Materials and methods

Experimental Sources

This experiment was used to observe the survival time of 15 mice in each group, the average operation time was 90 minutes, and the operation success rate was more than 90%. In the acute rejection heart transplantation model, the average survival time of allograft heart was (7.5 ± 0.37) days. In contrast, in the homologous transplantation group, the transplanted heart survived 10 days after transplantation (observation endpoint). In the chronic rejection model, the average survival time of allograft heart was (63.4 ± 4.37) days, while the transplanted heart in the homologous group survived 100 days after transplantation (observation endpoint). At each end of observation, the grafted heart was taken. Visual observation showed that the donor heart in the allograft group was dark red to white, with severe adhesion to the surrounding intestinal canal and other tissues, and it was hard and brittle to touch (16).

Experimental Procedure

First, donors cut 6-8 weeks Balb/c mice or Bm12 or C57BL / 6 mice as donors and C57BL / 6 mice as recipients. The mice were anesthetized with 0.5% pentobarbital sodium 0.3 ml, and 1 ml of normal saline containing heparin was injected through the inferior vena cava to make the mice heparinized. After 1 rains, open the chest cavity, expose the heart, and apply saline ice cubes to protect the myocardium (17). Use 5-0 mousse thread to ligature the upper and lower vena cava of the heart, cut the distal end of the heart with scissors, find the aortic arch, and separate it from the transverse aorta.

Secondly, after anesthetizing with 0.5% pentobarbital sodium, the skin was disinfected with 75% alcohol; a midline abdominal incision, intestinal mixing was carried out to the right, covered with sterile saline-soaked in normal saline, fully exposed the surgical field of vision, free left renal artery level Abdominal aorta and inferior vena cava bifurcating with the iliac artery, 11-0 silk thread was ligated to the lumbar vein, 5-0 mousse thread was ligated to the proximal end and the distal end respectively, and Venus scissors were used to inferior vena cava and abdominal The anterior wall of the main Egypt is cut with a longitudinal opening, which is consistent with the cardiopulmonary donor artery and the active caliber.

Finally, the model was evaluated, and abdominal palpation was performed every day to check the heart rate and pulse strength of the graft. The normal

pulsation for more than three days was successful. A graft heart specimen was prepared, and the mice were sacrificed after anesthesia. The graft heart was isolated and washed with physiological saline at the root of the aorta. The apex of the heart was placed in liquid nitrogen and frozen for QPCR analysis (18). 4% paraformaldehyde in the heart was prepared for tissue section analysis. According to the HE staining results, the arterial graft disease (GAD) score was evaluated based on the number and severity of vascular involvement in the epicardium and myocardium.

The Purpose of the Experiment

To clarify the role of miR-155 in heart transplantation, we performed Th17-related cytokine analysis on mice seven days after transplantation. We analyzed the T cell subsets of the spleens of both groups of mice and found that the CD4 + IL-17A + cell subsets were significantly reduced (19). Local QPCR of the transplanted heart also showed this phenomenon. The expression of IL-17A and IL-9 in the miR-155 receptor mouse group was significantly reduced, and the expression of Foxp3 was increased. Then, seven days after transplantation, the expression levels of plasma IL-9 and IL-17A were detected by ELISA, and only IL-17A expression was significantly reduced. Further analysis of IL-17A expression levels in the plasma of mice at various time points after transplantation found that IL-17A expression was significantly reduced at 3, 5, and 7 days after transplantation, suggesting that miR-155 knockout has a cardiac rejection effect. The protective effect may be closely related to the expression of IL-17A (20). In view of the high expression of IL-17A can recruit neutrophils and effector T cells to the local part of the graft, thereby accelerating the process of graft rejection. Therefore, we performed CD3 and Gr-1 immunohistochemical staining on the transplanted heart, and at the same time, isolated the local infiltrating cells for flow cytometry analysis. We found that in the miR-155 receptor mouse group, CD3 + and Gr-1 + cells in the transplanted heart were significantly reduced (21). The ratio of CD45 + CD11b + and CD45 + Gr-1 + is significantly reduced (22).

Results and discussion

As shown in Figure 1 (The data in the figure is collected by the author), there is no small difference in the expression of miR155 in different doses. In comparison, the high-dose group will significantly increase the miR-155 changes. In different experimental groups, different pharmaceutical tests were made according to the comparison, and the results obtained were also different. In the control group, the expression of miR155 changed only 2.1, which was the least cha among all groups, and it was also the result of no drug intervention. In the Bm12 dose group, the expression change of miR155 was 3.4, and the expression change of miR155 in Balb / c mice reached 6.8, and the expression change of miR155 was very high in the two comparison groups with the addition of the agent. 11 and the high-dose group reached 12.3. It can be seen from this that the effects of the agents are more obvious, and the two agent groups have changed significantly more than the other experimental and control groups.

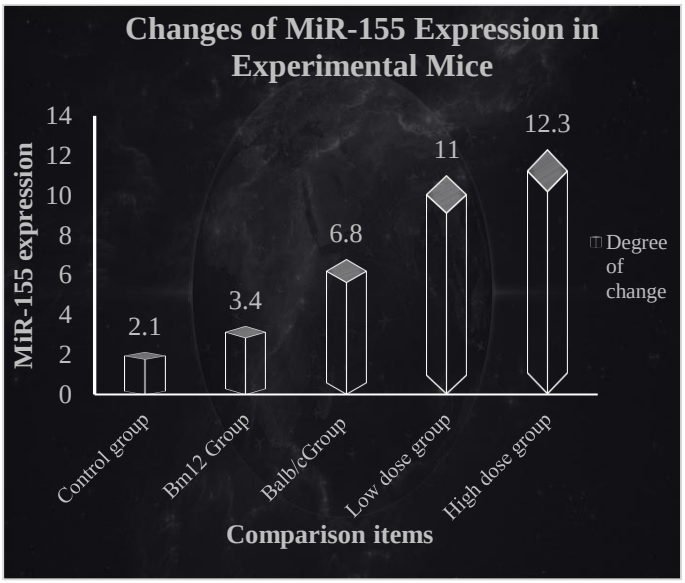

Figure 1. Changes of MiR-155 Expression in Experimental Mice

As far as the current technology is concerned, Th17 cells with miR-155 + / + and miR-155-/-partially isolated directly from the graft for the analysis of target gene chips are very difficult. However, many transcription factors associated with Th17 cell subsets have been shown to be miR-155 as one of the target genes. As shown in Figure 2 (Data from the website of the National Bureau of Statistics), these

transcription factors are involved in miR-155 regulating chronic graft rejection. The insignificant changes were STAT6, PU.1, Smad2 transcription factors. Under the miR-155 + / + and miR-155-/-two agents, there was no significant change in protein breakdown, which were 1.3, 0.8, and 1.2, respectively. The other two transcription factors, SOCSL and C-maf, are relatively obvious. Reached 4 and 2, respectively, with statistical significance (P <0.05). It can be seen that these two are consistent with the whole response, and provide strong support for the acute response of the two immune cells. Therefore, we can explore the role of miR-155 in the differentiation of Th17 cells. We performed relevant protein expression analysis on miR-155 + / + and miR-155-/- induced Th17 cells in vitro. From this we can conclude that a variety of miR-155 is involved in the regulation of heart transplantation.

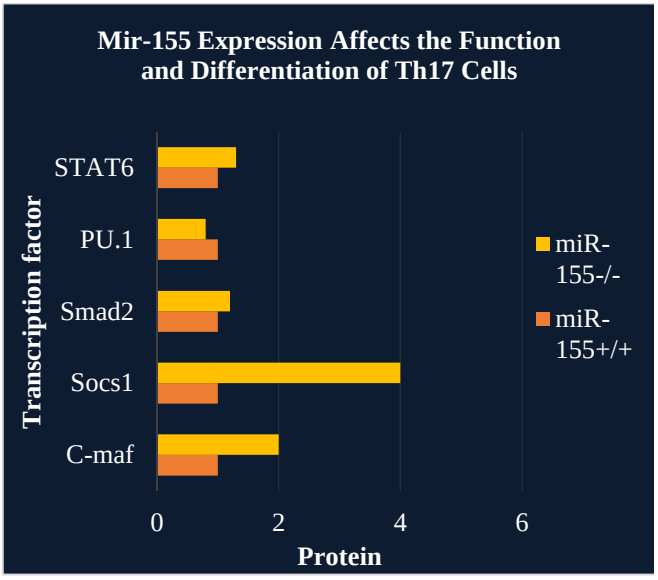

Figure 2. MiR155 Expression Affects Th17 Cell Function and Differentiation

Throughout the experiment, as the heart transplantation progressed, the weight of the mice changed. As shown in Fig. 3 (Pictures from *www.Wikipedia.com*). In the control group, the weight change of the mouse increased steadily from the initial 202 grams, experienced 314 grams, 321 grams, and finally weighed 420 grams. During the whole process, there was no external stimulus, so there was no abnormal change. The two experimental controls, Bm12 and Balb/c had some changes in body weight. The former is 210 grams, 332 grams, 285 grams and 380 grams, the latter is 220 grams, 332 grams, 285 grams and 380 grams. On the 24th day, the body weight decreased to varying degrees, and the weight of the two pharmaceutical groups was basically at a higher level. The body weight of the low-dose group was 215 grams, an increase of 355 grams, and then fell to 340 grams, and the final is 386 grams. The body weight of the high-dose group was 214 grams, 340 grams, 380 grams, and 370 grams.

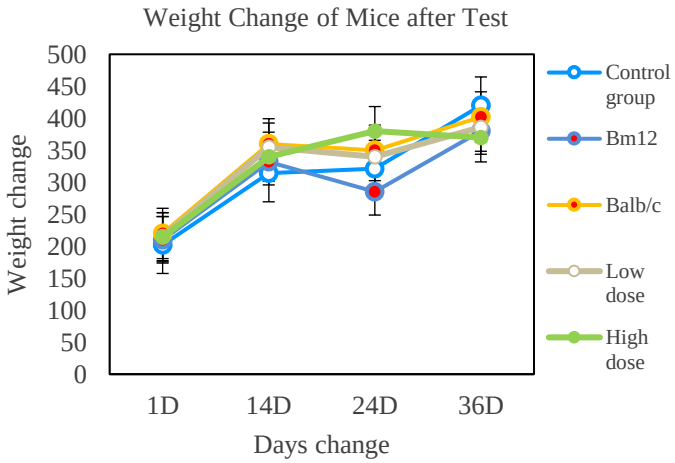

Figure 3. Changes in body weight in mice

As shown in Figure 4 (Pictures from *www.baidu.com*), HE staining of the mice in the control group showed that the internal epithelium of the tissue was flat. The pathological analysis found that in the acute rejection model of heart transplantation, many inflammatory cells infiltrated in the transplanted heart in the allogeneic group, with obvious myocardial fibrosis and necrosis, for severe acute rejection. In the chronic rejection model of heart transplantation, allograft heart myocardial tissue has a large amount of inflammatory cell infiltration around and around blood vessels, the cell wall is significantly thickened, and there is also moderate inflammatory cell infiltration in the tissue, with degeneration and necrosis of myocardial cells.

As shown in Figure 5 (The picture comes from the experiment of HowNet), the correlation analysis of the expression of MIR155HG and myostatin C in the serum of patients with chronic heart failure. Linear correlation analysis of the correlation between serum MIR155HG and left ventricular end-diastolic volume (LV-EDV) and left ventricular ejection fraction (LVEF) in 17 experimental heart transplant mice

showed that there was a positive correlation between MIR155HG and left ventricular end-diastolic volume y = -3.4957x + 54.591R² = 0.257 (P <0.001), and has no correlation with left ventricular ejection fraction. MIR155HG increased slowly at first and then continued to increase. The expression of serum MIR155HG in patients with ischemic or non-ischemic heart failure was significantly higher than that in experimental mice after heart failure.

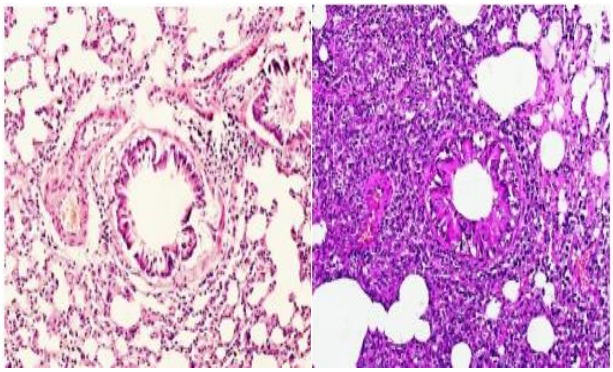

Figure 4. Comparison of Pathological Staining of Cardiac Tissue

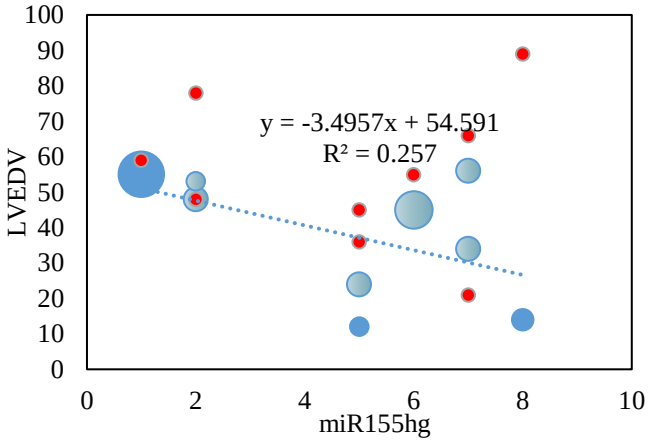

Figure 5. Correlation of serum miR155 expression with left ventricular end-diastolic phase

The mouse heterotopic heart transplantation model is an important method to study transplantation immunity. In recent years, with the wide application of a variety of transgenic and gene knockout mice, the progress of transplantation immunity research mechanisms has been greatly promoted. Mice have become the first choice for studying transplant rejection (23). Heterotopic heart transplantation in mice can usually be divided into two types according to the construction site. Abdominal heterotopic heart transplantation and cervical heterotopic heart transplantation (24). Compare the levels of cytokines in the hyperplasia and secretion phases with the control group. No matter in the hyperplasia or secretion phase, the levels of IL-10 in serum and intraperitoneal fluid are higher in patients with endometriosis than the control group, while the levels of IL-2 There was no significant difference between the groups (25). It shows that a large amount of IL-10 can reduce the phagocytosis of ectopic endometrium by inhibiting the activity of NK cells. At the same time, IL-10 also inhibits the production of Th1 cytokine IL-2, which prevents IL-2 from exerting its normal function and further aggravates the decline of NK cell function.

Regardless of the mouse heterotopic heart transplantation model, the coronary circulation path is the same, the recipient abdominal aorta-donor ascending aorta-transaortic root-coronary artery-myocardium-coronary vein-coronary sinus ostium-Right atrium-right ventricle-pulmonary artery-inferior vena cava. In this circulatory mode, the left heart system of the donor heart is empty and does not participate in blood circulation. The entire heart is equivalent to a pulsating foreign body organ. Therefore, this model is widely used to study the rejection mechanism of heart transplants (26). This experiment found that the success rate of the abdominal heterotopic heart transplantation model in mice is closely related to the proficiency of the surgeon in microsurgery. The mice were fully heparinized before donor heart removal, and the coronary arteries were fully lavaged after the removal to prevent microthrombosis in the coronary vessels, causing coronary artery blockage and myocardial infarction after transplantation. The anastomosis strictly guarantees vascular valgus anastomosis, avoids the adventitia and fat tissue to be brought into the intima, and prevents thrombosis after vascular anastomosis. Enlarging the venous anastomosis, because the venous blood flow is slow, it is easy to cause anastomotic stenosis and thrombosis. Maintain body temperature, maintain a temperature of 20-30 °C during the operation, and reheat in a 37 °C incubator after the operation. If the temperature is too low or too high, it will lead to different degrees of microcirculation disorders and fluid loss, ensuring the normal heart transition.

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Conflict interest

The authors declare no conflict of interest.

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