BAG2-activated cell autophagy and mir-27b dynamic regulation mechanism during *Mycobacterium tuberculosis* infection

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ARTICLE INFO

ABSTRACT

Tuberculosis is a highly contagious infectious disease. *Mycobacterium tuberculosis* infection is the main cause of tuberculosis. During the infection of *M. tuberculosis*, the expression of the resistance gene BAG2 will change, and miR-27b will play a certain role in dynamic regulation. The purpose of this article is to explore in-depth the effect of BAG2 on cell autophagy during *M. tuberculosis* infection and the dynamic regulatory mechanism of miR-27b on BAG2 activated cell autophagy. Fifty rats were used as experimental subjects, and *M. tuberculosis* strains H37Ra and H37Rv were implanted into the rats. Fluorescence quantitative PCR was used to detect the dynamic changes of BAG2 and miR-27b expression levels in rats and the regulatory effect of miR-27b on BAG2, and the effect of changes in BAG2 expression levels on cell autophagy was studied by immunoblotting. The results showed that after *M. tuberculosis*-infected macrophages, the expression level of BAG2 decreased from (284.24±6.31) to (156.48.24±4.49), and the expression level of miR-27b was increased from (43.72±3.35) to (78.35± 4.17), the apoptosis rate increased by 17.8%, and the autophagy rate increased by 20.6%. Therefore, it can be seen that the up-regulation of miR-27b expression level during *M. tuberculosis* infection will inhibit BAG2 expression, thereby promoting cell autophagy and apoptosis to reduce the survival rate of *M. tuberculosis*.

Introduction

Tuberculosis is a typical infection caused by an acute infection of *Mycobacterium tuberculosis*, and infection with a pathogen can cause the highest patient mortality. Today, the annual morbidity and mortality of chronic tuberculosis worldwide are increasing year by year, and recently it has become one of the ten major diseases of human health worldwide. *M. tuberculosis* is mainly present in the host macrophage granulocytes of tuberculosis-infected persons and can regulate the apoptosis process of macrophage granulocytes through various immune mechanisms. Studies have shown that BAG2 is involved in the regulatory mechanism of miR-27b in the inflammatory immune response caused by chronic *M. tuberculosis*, which can effectively reduce the viability of *M. tuberculosis*. Therefore, studying the regulatory mechanism of BAG2 and miR-27b is of great significance for the treatment of tuberculosis. *M. tuberculosis* has a strong ability to survive. It continuously stimulates the malignant inflammatory reaction of the infected tissue and gradually develops into tuberculosis. Gu found through research that not only *M. tuberculosis* can infect humans but also animals, and also pointed out. Tuberculosis mainly enters the host through the respiratory tract, but there are other ways of infection (1). In the article, Kumar analyzed the causes and infection process of *M. tuberculosis*, introduced some valuable prevention and treatment methods for tuberculosis, and briefly described the adverse effects of *M. tuberculosis* on the human body (2).). Nguyen found through research that tuberculosis can successfully host in host macrophages after infection with *M. tuberculosis*, the host body will automatically activate the immune system to reduce the growth and spread of the virus,

tuberculosis will evolve accordingly, avoiding the clarity of the host immune system proves that the human immune system can cure tuberculosis (3). Safi elaborated on the pathogenesis and pathology of tuberculosis in the article and analyzed the current research status and research methods of tuberculosis in my country (4). Through research, Fingelkurts found that BAG2 automatically activates cell autophagy during the infection of *M. tuberculosis*, at the same time, miR-27b can dynamically regulate the expression level of BAG2 and play a role in inhibiting the sensitive activity of *M. tuberculosis*(5). In summary, *M. tuberculosis* is the cause of tuberculosis, which is extremely infectious and difficult to cure. Cellular autophagy is a process that changes genes in cells during the evolution of eukaryotes. Whelan gave a detailed introduction to the mechanism of cell autophagy, analyzed the effects of cell autophagy on various tissue functions, and emphasized that cell autophagy is a normal manifestation of cell metabolism (6). Through research, Zhou found that cell autophagy is closely related to the changes of micro miR-gene expression during the infection of *M. tuberculosis*, pointing out that cell autophagy in tuberculosis lesion tissue can affect the gene regulation mechanism, thereby reducing the damage caused by tuberculosis to the human body (7). Through research, Reid found that cell movements such as autophagy, apoptosis, migration and value-added are the basis for cells to exert their biological functions through regulation, so cell autophagy can affect the living space and dynamic changes of *M. tuberculosis* (8). Drissi emphasized in the article that although there is no clear pathophysiological way to study tuberculosis, the researchers have proposed many related theories, including miR-27b dynamically regulating BAG2 to treat tuberculosis (9). Sohni emphasized the importance of cell autophagy, and analyzed the principle of cell autophagy, and pointed out that cell autophagy is an important method for the human immune system to defend against tuberculosis infection (10). It can be seen that cell autophagy is a normal response to human body function and can stabilize the immune system to a certain extent.

This article mainly explores the effect of BAG2 on cell autophagy during *M. tuberculosis* infection and the dynamic regulation mechanism of miR-27b on BAG2 activated cell autophagy. In this research

project, 50 rats were used as experimental objects. Mycobacterium nucleate strains h37ra and h37rv were implanted into the rats. The dynamic changes of BAG2 and miR-27b expression levels and miR-27b regulate BAG2. The results showed that after *M. tuberculosis*-infected macrophages, the expression level of BAG2 decreased from the original (284.24±6.31) to (156.48.24±4.49), and the expression level of miR-27b was increased from (43.72±3.35) to (78.35±4.17). The innovative focus of this research is to extensively use modern molecular and microbiology and electronic fluorescence detection and other advanced medical detection techniques to study the dynamic expression regulation and internal BAG2 expression of miR-27ba during the process of chronic tuberculosis cell branch coli entering infected cells the direct connection between the dynamic changes, and the direct effects of the changes in the expression of tuberculosis cells and BAG2 on the cytotoxic apoptosis of tuberculosis cells, endoplasmic reticulum cell autophagy inflammatory toxicity, and the importance of tuberculosis. It can be seen from this that this article studies *M. tuberculosis* from a new direction, improves the experimental effect, and provides a valuable reference for the treatment of tuberculosis.

Materials and methods
Mechanism of Cell Autophagy against *M. tuberculosis* Infection

Cellular autophagy metabolism is a dependent cell degradation metabolic process, which can effectively degrade various proteins and other organelles that lead to the damage of the body's functions, which is conducive to promoting the destruction of harmful substances in the body and effectively resisting the massive invasion of fungal viruses in vitro (11). As shown in Figure 1, the process of cellular autophagy can be subdivided into four main steps: Cell formation of natural macrophage precursors, cell formation of natural macrophages, cell formation of auto phagolysosomes, and autophagolysosomal-based the body's cells are degraded (12). Autophagy flow differentiation is the sum of the differentiation of the four main steps of autophagy in human cells. It is a dynamic differentiation process in which the four main steps of autophagy in human cells are continuous within the autophagy cells. Physiological

influences or physiological obstacles may occur, and autophagy cannot perform all its functions normally (13).
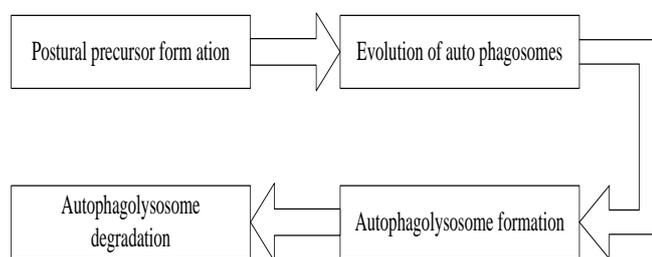
Figure 1. Cellular autophagy can be divided into four steps

Mycobacterium tuberculosis (MTB) spreads mainly through the alveolar channels to the infected body. When invading an infected body, it is inhibited and absorbed by alveolar macrophages leukocytes that can directly enter multiple nerve organs of the infected body (14). Macrophages are not only the main target cells of human MTB but also the main immune stress and protective immune cells of the human body (15). After the virus invades the host, the macrophage system will be associated with immune response mechanisms (such as apoptosis, autophagy, necrosis, signal transduction pathways (such as TSX and TXP) and mediated host inflammation and immune response). Studies of immune mechanisms such as autophagy generally believe that they are beneficial to the host's inflammatory defense, while studies of immune mechanisms such as necrosis often indicate that they are a continuation of other pathogens (16). Autophagias can directly participate in the interaction between MTB cells and macrophage receptor cells in vivo, which is beneficial for the in vitro clearance of BCS and MTB in vivo. Cellular autophagy rate is a key factor in determining the activity of the tuberculosis virus (17). The calculation formula of macrophage autophagy rate after mycobacterium tuberculosis infection is shown in 1.

$$X_j^l = f\left(\sum_{i \in M_i} X_j^{l-1} * K_{Ij}^l + b_j^l\right) \qquad (1)$$

Among them, X represents the autophagy rate of macrophages, K represents the activity of *M. tuberculosis* virus, and b represents the cell operating factor.

After the *M. tuberculosis* virus invades the human body, macrophage immune cells are recruited to the main infectious immune area and are activated by co-stimulating human airway mucosal epithelial macrophages and p-THK-α macrophage factors. Macrophage receptors recognize immunoreceptors (PRRS) through their cell surface patterns and can recognize the main pathogens of tuberculosis and related molecular recognition patterns (18). Macrophage autophagy can also recognize other tuberculosis through the above-mentioned PRR molecular family chain, start cell autophagy, and effectively resist the continuous invasion of other tuberculosis (19).

Object selection

In this experiment, 50 rats with close to six months of body weight were selected as the research object, and the laboratory that had been disinfected and non-polluted was selected as the experimental site. The temperature of the laboratory was 24-28°C, and the moisture in the air was about 17%-20%, to ensure that the laboratory has a good daylighting rate, to be reared in accordance with the national regulations for feeding, and to exclude unhealthy rats before the experiment.

Related materials

Main materials: *M. tuberculosis* strains h37ra and h37rv, rat macrophage cell lines j744 and raw264. Main instruments: Agarose level electrophoresis instrument, lpm electric thermostatic water bath, sys fluorescence quantitative PCR instrument, RNA centrifuge tube, transmission electron microscopy, VST multifunctional microplate reader, fluorescence measurement instrument ppl, low-temperature centrifuge, s-8000 overspeed centrifuge, chemical reaction luminometer, kd-520 transfected cell incubator, XCN pipette, biochemical incubator, gene activity detector, biological safety cabinet, magnetic stirrer.

Main reagents: Tabs reverse transcription kit, transfection reagent 3000ml, KST reverse transcription reagent 400ml, raz-1028 medium 500ml, fetal bovine serum 230ml, dual luciferase detection

reagent 320ml, medical absolute ethanol 2000ml, protein extraction reagent 160ml, methanol, etc.

Strain culture and model establishment

H37rv colony inoculation is diluted with 0.5ml fetal bovine physiological chlorinated saline and stung fetal bovine culture solution using a mixed solution (bacterium species volume ratio of 2:3) to mix and dilute. Each BCG fetal bovine vaccine is used with fetal bovine special dilution 0.5ml mix and dilute, inoculate the inclined surface of rotche's medium, and cultivate for 15 days, select each fetal cow h37rv and each BCG colony with good growth and development status, and use each fetal cattle physiological chloride containing 0.25% tween-50 the brine is ground evenly (20). Mix it with a good deem and culture medium (using 20% fetal bovine serum) to make a fine bacterial suspension micro-suspension. The adjusted bacterial suspension concentration is 2×10^7/ml. Dmraw264.7 cell staining was placed in demo culture medium for culture, digested with 0.85% phosphorescing and collected tumor cells, using live trypan blue staining method for live tumor cell staining detection, staining and counting under the microscope after adjustment, the viable cell staining concentration was 5.0×10^7 cells/ml (21). The cultured rat tuberculosis cell mycobacterium strain cells were injected into a rat body to facilitate the establishment of a rat tuberculosis cell mycobacterium strain infection cell model (22).

Construction of BAG2 eukaryotic expression vector

Design primers according to the template of the enzyme in fBAG2 in NCB, select PCDNA from the rat bacterial macrophage cell lines j744 and fcraw264 as the design template to perform the shaker PCR reaction on the enzyme and determine the recovery of the specific shaker band according to the length of the band. At 32°c, shake the enzyme with the rotary cut overnight fragment and shake the carrier for pcdna3. The reaction condition was 14°c. Shake the bacteria to continue the ligation overnight, after conversion into the enzyme dh5 competent cells into the cell, coated with the solid medium in LB (amp has (resistance), select and extract a single enzyme clone and shake at 32°c overnight to connect the shaker, save a part of the shaker solution and no longer extract the plasmid,

the enzyme is identified by rotary cutting or fluorescence electrophoresis, and the success of the construction is determined according to the sequencing results (23).

RNA reverse transcription

In this experiment, TADBS reverse transcription kit was used for virus infection DNA synthesis. Take 3μg of micro RNA from the sample as a template for RNA reverse transcription to generate DNA. As shown in Table 1, the relevant parameters of the reverse transcription system determine the rate of reverse transcription generation.

Table 1. RNA reverse transcription system; Group Name (A), Volume required for 20μL system (B), and Reaction time(h) (C)

A	B	C
5×PrimeScript Buffer	4μL	4.6
Prime Script RT Enzyme Mix I	1μL	3.8
Random 6 mars(100μM)	2μL	4.9
Total RNA	2μL	5.2
RNase Free ddH2O	10μL	8.3

Detection of BAG2 and miR-27b expression levels

Use an electronic fluorometer to quantify per to detect the levels of expressions such as aBAG2, miR-27br (24). The PCR molecular factor combination reaction can continue to be carried out quickly on the BAG2 and miR-27bca factor combination reaction coenzyme amplifier. The content of the factor solution in the 100μl reaction coenzyme template system includes: 5×pcr buffer, upstream primers and downstream primers 0.35μmol/ l, DNA factor reaction coenzyme template is about 800ng, tangoed, the connection distance between the coenzyme body and the base layer of each reactant is set to about 4μm, and the expression level of BAG2 and miR-27b can be determined by the pasteurized agarose concentration detection analyzer (25).

Results and discussion

Results of Endoplasmic Reticulum Stress-Induced by *M. tuberculosis* Infection

After the rats were infected with *M. tuberculosis* h37rv and h37ra, the specific response of endoplasmic reticulum stress was detected by immunoblotting. The results of the study showed that during the infection of *M. tuberculosis* in rats, hspa2 and chop in the endoplasmic reticulum increased. As shown in Table 2, as the infection time and the degree of infection increase, the expression of hspa5 and chop will gradually increase, which means that the endoplasmic reticulum of rat viral cells is activated, which in turn triggers the activation of autophagy.

Table 2. HSPA5 and CHOP expression will gradually increase; HSPA2 (A), CHOP (B), and Protoplasmic (C)

Group	After 6 hours	After twelve hours	After twenty-four hours	X2
A	79.63±4.22	110.47±5.58	238.24±8.55	11.36
B	88.12±5.07	136.84±6.27	247.16±10.02	9.57
C	72.36±4.08	155.07±7.27	281.75±9.38	8.68

Effect of *M. tuberculosis* Infection on miR-27b Expression

The results of the study showed that after infection with *M. tuberculosis* in rats, the expression level of miR-27b in the cells will be increased. As shown in Figure 2, the expression level of miR-27b was detected by quantitative PCR, and the expression level of miR-27b in macrophages was found. It was increased from (43.72±3.35) to (78.35±4.17), indicating that the *M. tuberculosis* virus can promote the expression of miR-27b.
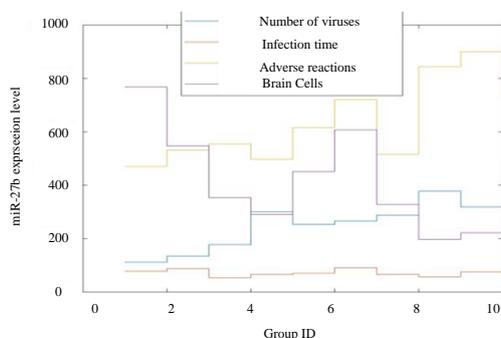
Figure 2. Effect of Mycobacterium infection on the expression of miR-27b

Effect of miR-27b on Inflammatory Factors under Infection

The results of the study showed that during the infection of *M. tuberculosis* in rats, as the expression level of miR-27b was up-regulated, the activity of intracellular inflammatory factors will be reduced. As shown in Figure 3, after injection of *M. tuberculosis* strains in rats, the intracellular inflammatory factor activity index decreased from the original (1425.33±20.58) to (1182.49±15.37), proving that the up-regulation of miR-27b expression level has an effect on the activities of inflammatory factors il-1β, il-6 and TNF-α certain inhibitory effect.
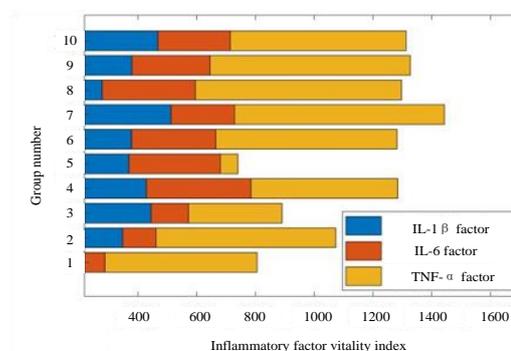
Figure 3. Effect of miR-27b on inflammatory factors under infection

Dynamic Regulation of BAG2 by miR-27b under Infection

The results showed that with the increase of miR-27b expression level, the expression level of BAG2 in rat cells decreased. As shown in Figure 4, under the dynamic regulation of miR-27b, the expression level of BAG2decreased from the original (284.24±6.31) to (156.48.24±4.49), proving that miR-27b can effectively inhibit the expression of BAG2.
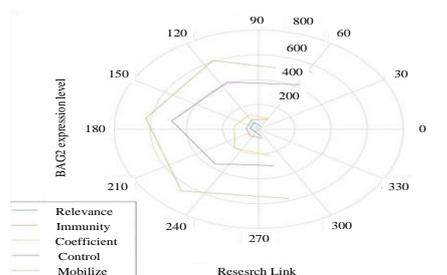
Figure 4. Dynamic regulation of BAG2 by miR-27b under infection

Effect of BAG2 on Cell Autophagy under Infection

The study found that during the infection of *M. tuberculosis* in rats, the dynamic regulation of BAG2 expression by miR-27b increased the apoptosis rate and autophagy rate in rats. As shown in Figure 5, the apoptosis rate increased by 17.8%, the rate of cell autophagy increased by 20.6%, proving that BAG2 has a certain inhibitory effect on cell autophagy, and the decrease in BAG2 expression stimulated the increase of cell autophagy activity.
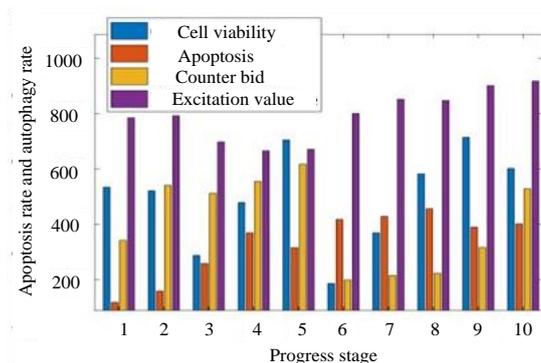
Figure 5. Effect of BAG2 on cell autophagy under infection

Acknowledgments

None.

Interest conflict

The authors declare no conflict of interest.

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