



# miR-126 ameliorates multiple organ dysfunction in septic rats by regulating the differentiation of Th17/Treg

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## Abstract

**Background** The most common cause of death in sepsis is MODS. We hope that miR-126 can regulate the differentiation of Th17/Treg, reduce the infiltration of inflammatory factors in peripheral blood and various organs and tissues, and improve organ function and prognosis in sepsis.

**Methods and results** Septic rat model was established by cecal perforation and ligation. miR-126 mimic and inhibitor were used to intervene sepsis. The experimental results showed that miR-126 mimic reduced the differentiation of Th17 and increased the differentiation of Treg in septic rats, resulting in the TNF- $\alpha$ , IL-6 and IL-17 were decreased in peripheral blood, the infiltration levels of TNF- $\alpha$ , IL-6 and IL-17 were decreased in lung, liver and kidney, the tissue damage degree of lung, liver and kidney were weakened, and the corresponding histopathological score decreased. Finally, the survival rate of septic rats was increased. However, after using miR-126 inhibitor, the levels of inflammatory factors and the degree of multiple organ injury in septic rats increased in varying degrees, and the prognosis of septic rats was worse.

**Conclusion** This study confirmed that miR-126 can regulate the differentiation of Th17/Treg, change the infiltration of inflammatory factors in peripheral blood, lung, liver and kidney of septic rats, alleviate MODS, and improve the organ function and prognosis of septic rats.

**Keywords** Sepsis · MODS · miR-126 · T Lymphocyte subsets · Pathological

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## Introduction

Infection has always been one of the major threats to human beings. Different from chronic diseases, infection associated diseases have the characteristics of rapid onset, disability and high mortality [1]. For example, cancer can leave us a period of time and opportunity to analyze and treat it, while infectious diseases can lead to the rapid deterioration of the patient's condition in a short time, and even we have no time to treat and intervene it. Most of the clinical infections come from viruses and bacteria [2], including atypical pathogens [3]. At present, the world's pandemic new coronavirus 19 is a serious threat to human health, and bacterial infection, especially sepsis caused by severe infection, also has a high mortality rate [4], and most viral infections will be followed by severe bacterial infection. Therefore, sepsis is still one of the major diseases in clinical infection, and the in-hospital mortality rate is as high as 26% [5].

With the further study of sepsis, the definition of sepsis has developed from the traditional 2.0 SIRS definition to the current 3.0 definition. The main difference is that version

3.0 adds the SOFA score, which is the evaluation of organ dysfunction [6]. The introduction of SOFA score to define sepsis is due to the pathophysiological mechanism of sepsis. In the initial stage, sepsis is the infection of the body, and then gradually evolved into one or more organ damage, until multiple organ dysfunction [7], so the final cause of death in sepsis is mostly caused by multiple organ dysfunction. Although organ dysfunction eventually occurred, it was only the result of sepsis development, and the main reason of MODS was the change of immune balance. Early immune function was extremely high, immune cells produced a large number of inflammatory factors, leading to immune storm [8]. Immune storm further causes the immune infiltration of tissues and organs so as to damage many organs, so it is very important to regulate the immune balance and inflammatory imbalance in sepsis [9].

MicroRNAs (miRNAs) are a class of small RNA molecules. By combining with target genes, they play the role of gene silencing and inhibiting gene translation. miRNAs are important in the progress of various diseases [10]. Now, it has been found that miRNAs have important significance for immune regulation, not only as a monitoring point of immune function, but also as an evaluation index of clinical prognosis [11]. As we all know, immune function is divided into innate immunity and acquired adaptive immunity. In terms of innate immunity, miR-126 is the first miRNA to participate in the host's reactive pretreatment to pathogen infection in a stable state. It is suggested that miR-126 may be a potential target for the treatment of infection, tumor and autoimmune diseases [12]. In adaptive immunity, the combination of miR-135 and miR-126 can inhibit the specific immune response (Th2). Overexpression of miR-126 can be used to regulate immune response (Th1 /Th17), and regulate the ability of immune signal transduction and immune cell migration [13]. A study has shown that the 24-h miR-126 level in patients with sepsis is significantly down-regulated, and the down-regulation of serum miR-126-3p is related to the severity of sepsis [14]. In asthma research, miR-126 is positively correlated with the percentage of Th17 cells in serum and the severity of asthma, while the CD4 + CD25 + Treg is negatively correlated with the severity of asthma, which proves that miR-126 has important predictive value for the diagnosis of asthma, and also indicates that miR-126 is related to Th17/Treg function [15]. Relevant studies have found that miR-126 can inhibit LPS induced the levels of HMGB1 in human microvascular endothelial cells. Overexpression of miR-126 can prevent microvascular disorders and improve the outcome of sepsis [16].

The purpose of our paper is that we changed miR-126 to intervene the differentiation of T lymphocyte subsets, and further intervene the levels of inflammatory factors released by lymphocytes, in order to reduce the infiltration of inflammatory factors in important organs and reduce organ damage

in sepsis. At the same time, we also observed whether miR-126 can evaluate the degree of inflammation and prognosis of sepsis.

## Materials and methods

### Animal model of sepsis

This research was approved by the animal ethics committee of Bengbu Medical College (Approval Number: 2018074). The arrangement and treatment of experimental rats in the study followed the ethics of experimental animals. The final euthanasia method of rats was to inhale 40% carbon dioxide until the rats were observed without any reaction such as breathing and heartbeat. The sepsis animal model was established by cecal perforation and ligation [17]. SD rats (200–250 g) were selected to make animal models and anesthetized with pentobarbital (20 mg/kg) via tail vein [18]. The animals were divided into four groups: Normal control group (NC), sepsis group (sepsis), sepsis rat + miR-126 mimic group (sepsis rat + miR-126 mimic) and sepsis rat + miR-126 inhibitor group (sepsis rat + miR-126 inhibitor). In vivo transfected mimics and inhibitors were injected via tail vein once a week for two weeks.

### Pathological score of organs

The lung, liver and kidney were selected for pathological observation of sepsis rat model. Paraffin embedded organ specimens were fixed with xylene and anhydrous ethanol after sectioning and washed with PBS. Hematoxylin eosin staining kit (KGA224, Jiangsu Kaiji Biotechnology Co., Ltd.China) was used for HE staining, drying, sealing and microscopic examination (All pictures were taken at 400X); After he staining, the sections were read by pathological professionals. Pathological evaluation criteria: according to the edema, structural changes, inflammatory cell infiltration of various organ lesions, from light to heavy, it can be divided into 4 degrees of 0–4 points. Finally, according to the total score calculation statistics.

### PCR analysis of inflammatory factors in tissues and organs

Lung, liver and kidney tissues were homogenized in PBS, and centrifuged by centrifugation. The supernatant was collected, and chloroform and isopropanol were added to separate the RNA in the supernatant. Realtime PCR was performed with a SYBR green assay (Japan.TaKaRa RR086B) using the ABI system (ABI PCR system USA.) The primers listed in Table 1. The relative changes in mRNA expression were determined by the relative quantitative method ( $-2^{\Delta\Delta Ct}$ ).

## Lymphocyte transfection and lymphocyte isolation count

Lipofectamine 3000 (Lipo3000, Invitrogen L3000015, USA) was used as the transfected liposome. miR-126 mimic or inhibitor was injected into the tail vein of rats for transfection. The peripheral blood of the animal model was taken and the lymphocytes were separated by density gradient centrifugation. Flow cytometry was used to count the collected lymphocytes. The lymphocytes were incubated at room temperature with fixed solution. The lymphocytes were labeled with CD4 (ebioscience 11-0040-82, USA), CD25 (ebioscience 12-0390-82, USA), IL-17 (ebioscience 12-7177-81, USA), Foxp3 (ebioscience 35-5773-82, USA). Finally, flow cytometry (Becton Dickinson FACS Calibur, USA) was used, The lymphocyte subsets were separated and counted.

## Detection of inflammatory factors

The blood of rats was collected and centrifuged. The upper serum of centrifuged blood was collected. The serum inflammatory factors (IL-6, IL-17 and TNF- $\alpha$ ) were determined by using rat ELISA kits (kgerc170-1, kgerc102a-1, kgerc003-1, Kaiji Biotechnology, China) for reaction coloration, and then the concentration of inflammatory factors was determined by using microplate (MD SPECTROMAX m3, USA).

## Statistical analysis

We used spss 24.0 software to process data. T-test or one-way ANOVA was used for comparison between groups, and Pearson correlation was used for correlation analysis. 95% confidence interval (CI) was used in all trials,  $p < 0.05$ , the difference was statistically significant.

## Results

### Pathology and prognosis of multiple organ dysfunction in septic rats

We found that the lung, liver and kidney of sepsis rats had serious pathological changes compared with normal rats. In the lung, there was mild congestion and thickening of alveolar wall, a small amount of secretion and obvious infiltration of inflammatory cells (Fig. 1a). In the liver, there

were obvious degeneration and necrosis of hepatocytes, mild dilation and congestion of hepatic sinuses, and slight infiltration of inflammatory cells in lobules and portal area (Fig. 1a). There were moderate edema of renal tubular epithelial cells, mild congestion of renal interstitium and infiltration of inflammatory cells in kidney interstitium (Fig. 1a). In addition, all the pathological scores in the sepsis group were higher than those in the NC group (Fig. 1b), and the mortality was also increased (Fig. 1c).

### Th17/Treg differentiation and inflammatory factor release in sepsis rats

T-lymphocytes can produce a large number of inflammatory factors during sepsis, which leads to immune storm, immune infiltration of tissue and organs, and multiple organ damage [19]. Therefore, we measured TH17/Treg in peripheral blood of septic rats and found that Th17 subsets increased and Treg subsets decreased (Fig. 2a). The differentiation of Th17 and Treg subsets lead to the excessive release of inflammatory factors in the serum of septic rats. The results showed that compared with NC group, TNF- $\alpha$ , IL-6 and IL-17 in serum of sepsis group were increased, and the infiltration of these inflammatory factors in lung, liver and kidney tissues were increased (Fig. 2b). Through the correlation analysis of inflammatory factors in peripheral blood and the degree of organ damage, as well as inflammatory factors in organs, we found that the inflammatory factors in serum and each organ damage (histopathological score) were positively correlated (Fig. 2c). In addition, the inflammatory factors in peripheral blood were positively correlated with the infiltration of inflammatory factors in lung, liver and kidney in sepsis (Fig. 2d). These results indicate that the Th17 / Treg can effect inflammatory factors in serum, lead to inflammatory infiltration of organs, and eventually lead to organ injury.

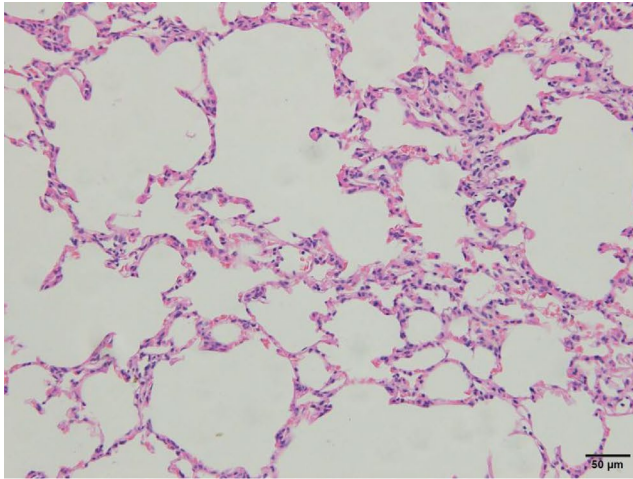
### miR-126 changed the differentiation of Th17/Treg

In order to change the inflammatory response mediated by the differentiation of Th17/Treg, we transfected lymphocytes in septic rats. After transfection of miR-126 mimics and inhibitors, we found that the expression of miR-126 was changed in T lymphocytes (Fig. 3a), and the differentiation was changed of Th17/Treg, which was consistent with our previous study, Induction of Th17/Treg differentiation through apoptosis pathway can change the inflammatory

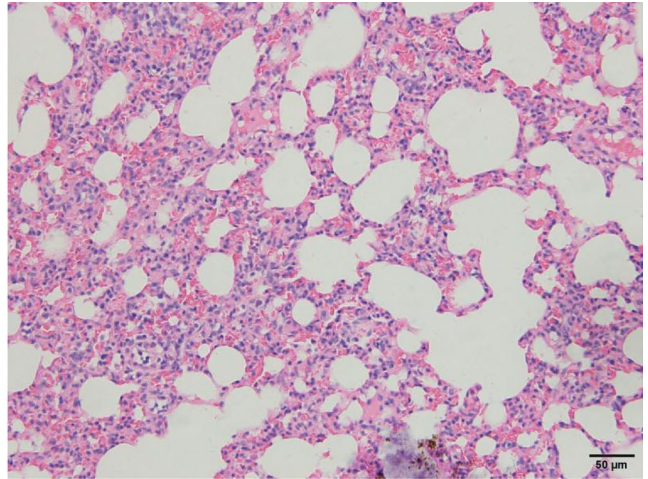
**Table 1** Primer sequences

Primers	Forward (5'-3')	Reverse (5'-3')
TNF- $\alpha$ mRNA	CCAGGATGAGGACCCAAGCA	CCAGGATGAGGACCCAAGCA
IL-6 mRNA	GCGGCAGATAAAAAGACTGC	GCAGCCTTCCTTCGTGTAAC
IL-17 mRNA	CTTCACCCTGGACTCTGAGC	TGGCGGACAATAGAGGAAAC

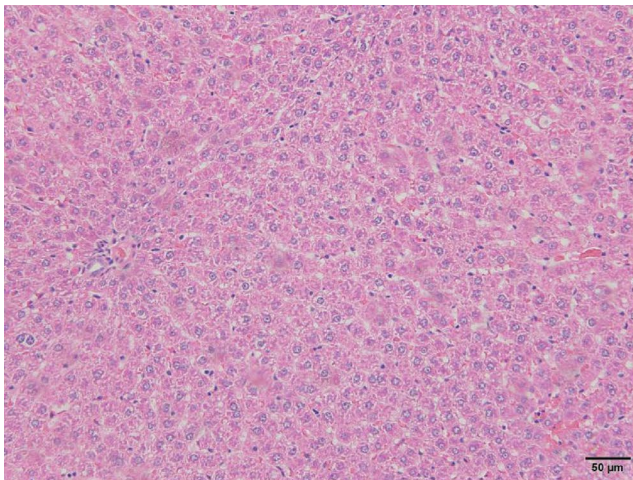
a



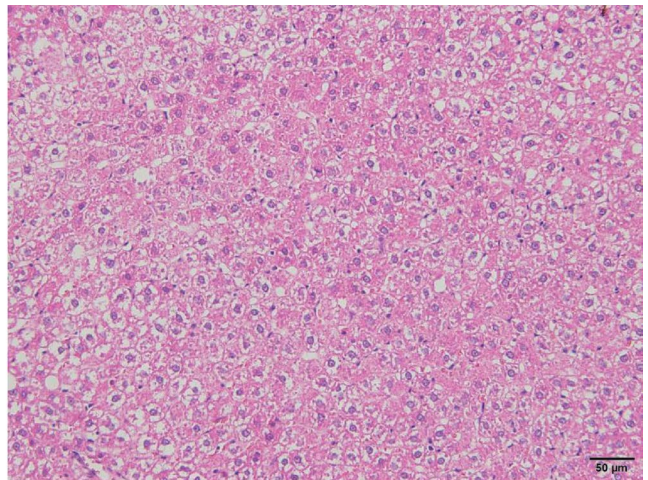
NC(Lung)



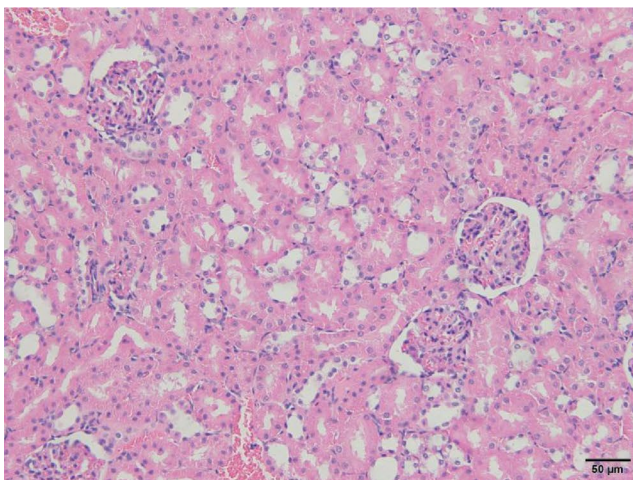
sepsis(Lung)



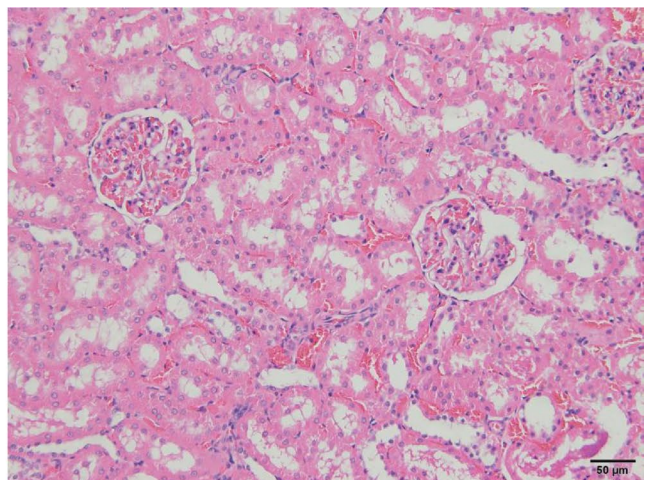
NC(Liver)



sepsis(Liver)



NC(Kidney)



sepsis(Kidney)

**Fig. 1** Compared with normal rats, sepsis rats have multiple organ dysfunction and poor prognosis. **a** Comparison of pathological sections of tissue (lung, liver and kidney) between normal rats and septic rats. **b** Comparison of pathological scores of lung, liver and kidney

between normal rats and septic rats.  $n=8$ ,  $**p<0.01$ . **c** The survival was recorded at different intervals. Each group contained 8 rats. Statistical significance was assessed by Log-Rank test and represented as follows:  $*p<0.05$  vs. NC. NC, normal control

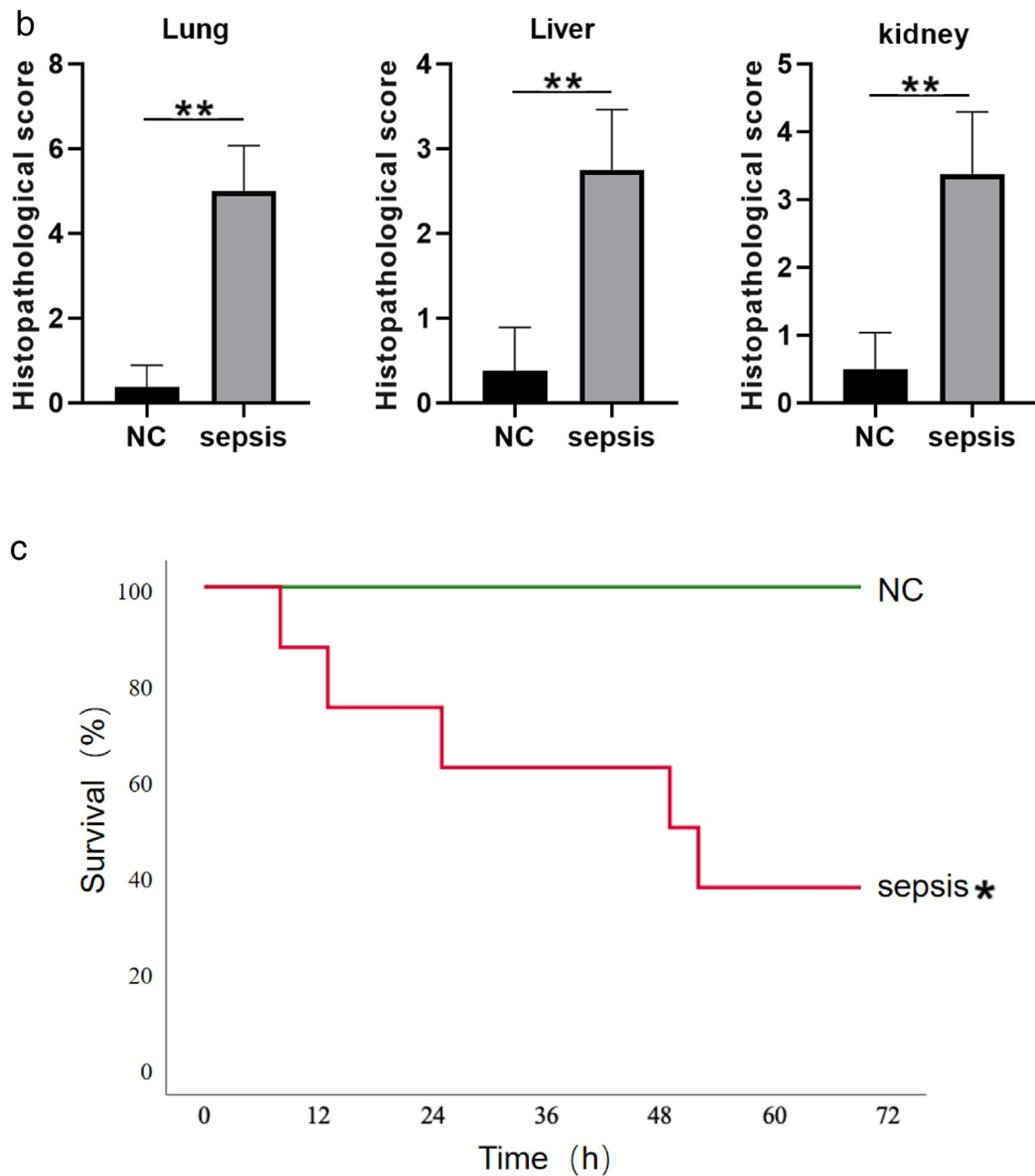


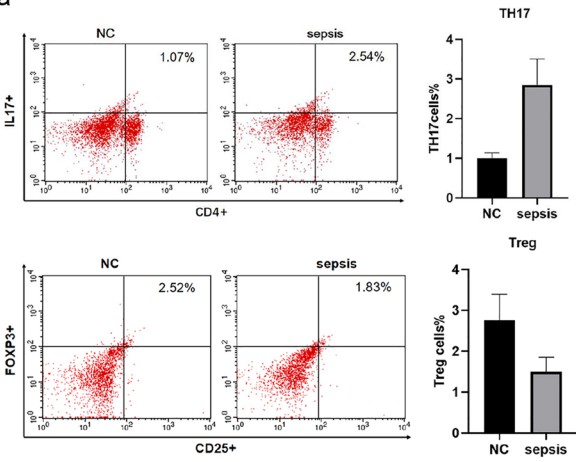
Fig. 1 (continued)

factors in septic rats [20]. We analyzed the Th17 and Treg in peripheral blood of septic rats after miR-126 intervention. The results showed that the Th17 in the mimic group were lower than those in the sepsis group, while the Th17 in the inhibitor group were higher than those in the sepsis group (Fig. 3b, c). However, in the group of Treg, the cell subsets of Treg in the mimic group were higher than those in the sepsis group, while the cell subsets of Treg in the inhibitor group were lower than those in the sepsis group (Fig. 3b, c).

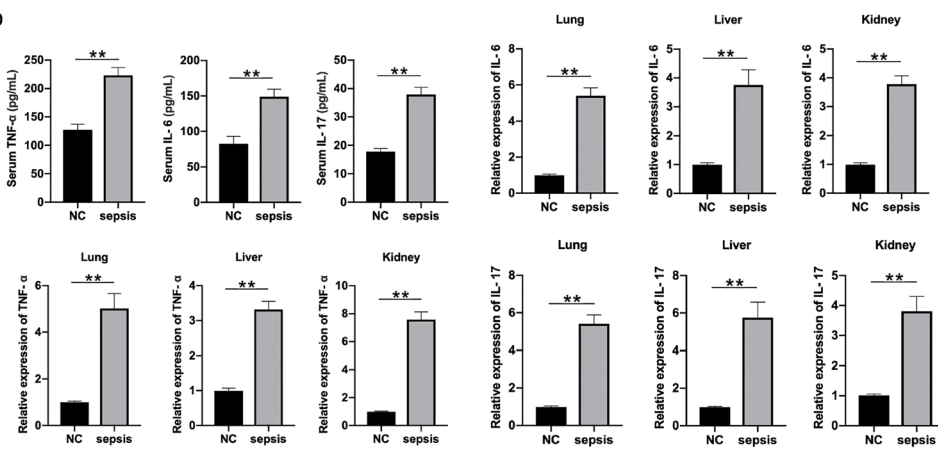
### miR-126 attenuates inflammatory infiltration in sepsis organs through Th17/Treg differentiation

Because the Th17/Treg subsets of septic T lymphocytes changed after miR-126 intervention, we further analyzed the inflammatory factors in organs and serum of septic rats after miR-126 transfection. The results showed that TNF- $\alpha$ , IL-6 and IL-17 in lung, liver and kidney in septic mimic group were lower than those in sepsis group (Fig. 3d). TNF- $\alpha$ , IL-6 and IL-17 in lung, liver and kidney in the sepsis inhibitor

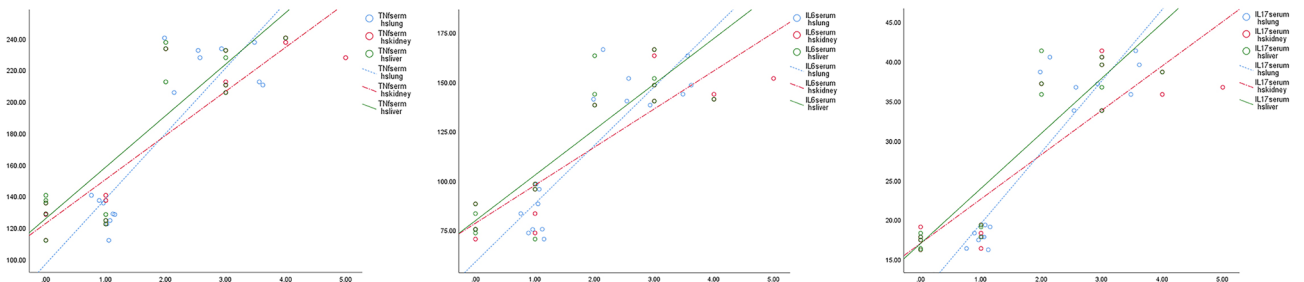
**a**



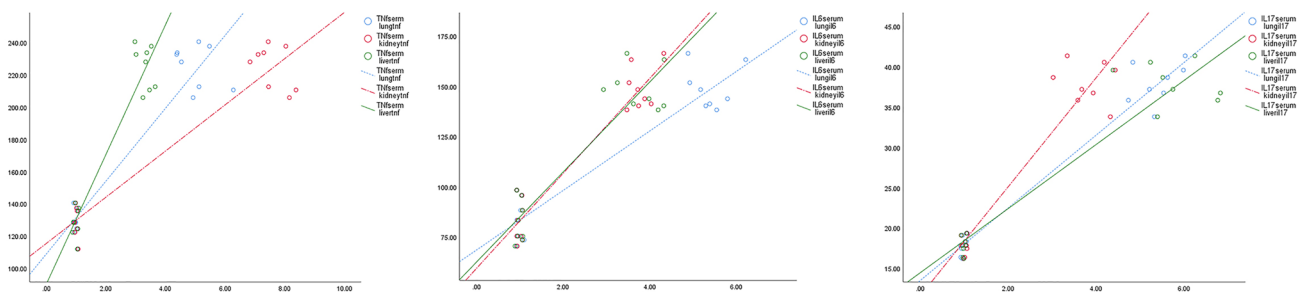
**b**



**c**



**d**



**Fig. 2** The changes of T lymphocytes and the expression of inflammation in peripheral blood and organs of rats. Correlation between inflammatory factors and histopathological score. **a** Comparison of Th17 and Treg subsets in normal rats and sepsis rats. **b** The levels of TNF- $\alpha$ , IL-6 and IL-17 in peripheral blood were compared between normal rats and sepsis rats. The expression levels of TNF- $\alpha$ , IL-6 and IL-17 in lung, liver and kidney were compared between normal rats and septic rats. **c** Correlation analysis between the release of inflammatory factors in peripheral blood and histopathological injury score. **d** Correlation analysis of inflammatory factors release level in peripheral blood and infiltration of inflammatory factors in organs. *NC* normal control

group were higher than those in the sepsis group (Fig. 3d). These findings were consistent with the release levels of TNF- $\alpha$ , IL-6 and IL-17 in peripheral blood (Fig. 3d). These results demonstrate that miR-126 can reduce the expression of inflammatory factors in serum and the infiltration of inflammatory factors in organs.

### miR-126 attenuates inflammatory factor mediated multiple organ dysfunction in sepsis

Because we found that the release of inflammatory factors in serum and organs could be decreased by T lymphocyte transfection of miR-126 mimics and inhibitors, we further analyzed the pathological changes of organs after miR-126 intervention. We found that the degree of pathological damage of lung, liver and kidney in sepsis mimics group was less than that in sepsis group (Fig. 4a). In sepsis inhibitor group, the pathological damage of lung, liver and kidney was more severe than that of sepsis group (Fig. 4a), and the pathological score was also increased (Fig. 4b). The survival rate of rats in the mimic group was higher than that in the sepsis group, while that in the inhibitor group was lower than that in the sepsis group (Fig. 4c). These results indicate that inflammatory factors are over released by septic inflammatory storm, which can lead to organ dysfunction. miR-126 can improve organ damage and improve the survival rate of septic rats.

### Relationship between organ damage and inflammatory factors infiltration in sepsis after miR-126 intervention

According to the above-mentioned expression of inflammatory factors and organ damage results, we consider that these situations may be due to the imbalance of Th17/Treg caused by sepsis hyperimmunity, which tends to Th17. Thus, these Th17 further promote the inflammatory infiltration of organs. Therefore, we further analyzed the correlation between T lymphocyte subsets and histopathological scores, and found that whether miR-126 mimic or inhibitor was transfected into septic rat, the release level of inflammatory factors in serum was positively correlated with

the pathological scores of organs in lung, liver and kidney (Fig. 5a). The release of inflammatory factors in serum was positively correlated with the infiltration of inflammatory factors in lung, liver and kidney (Fig. 5b). The results show that miR-126 can affect the release of inflammatory factors by changing the Th17/Treg, and alleviate multiple organ injury in sepsis.

## Discussion

At present, the final cause of death with sepsis is mostly due to multiple organ dysfunction, which makes more than two organs dysfunction and endangers life [21]. Although there are a series of organ support measures in ICU, such as ventilator, renal replacement therapy machine, and even extracorporeal membrane oxygenation (ECMO), it is difficult to curb the multiple organ dysfunction caused by sepsis without treatment from the cause of sepsis. As we all know, the core theory in the pathogenesis of sepsis is the inflammatory storm, which is the uncontrolled excessive release of inflammatory factors caused by the imbalance of immune function [22].

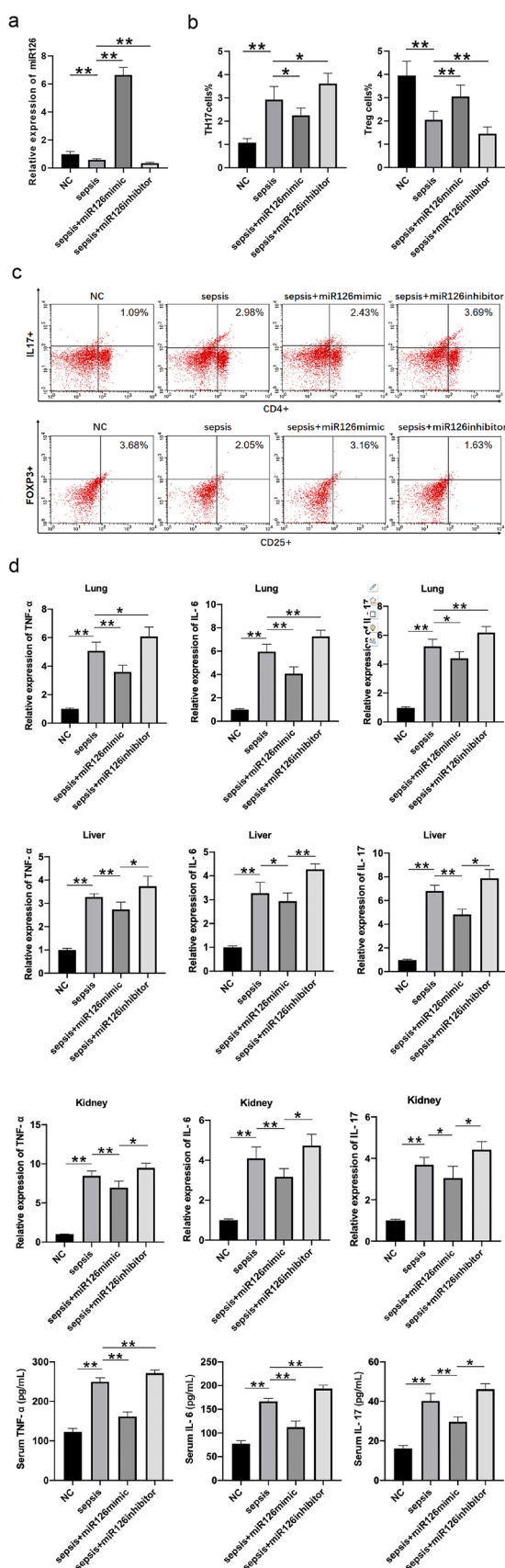
At present, there are three models of sepsis. (1) CLP rat cecal perforation ligation model, which has positive infection and heavy inflammatory reaction, but has the disadvantages of many types of intestinal bacteria, large individual differences of infectious pathogens and large individual differences of inflammatory reaction. (2) Intraperitoneal injection bacterial model has the advantage of single determination of bacteria, but the disadvantage is that each bacterial reaction is different and different from clinical infection. (3) The advantage of LPS model is that it can trigger the release of specific inflammatory factors and simulate the clinical inflammatory response, but the disadvantage is that unlike bacterial infection, it can simulate the septic response caused by some specific bacteria. This experiment is mainly to study MODS caused by inflammatory storm in sepsis, so LPS model is selected to simulate clinical inflammatory storm.

In MODS, overexpression of inflammatory factors is considered to be one of the core causes of organ dysfunction [23]. It has been reported that the initial role of these inflammatory factors is to resist the infection of pathogens in sepsis, but excessive release of inflammatory factors will cause damage to the body, such as acute lung injury, acute kidney injury, acute liver injury and so on [24]. So many studies are revealing the release and change of inflammatory factors. Our research focuses on how to improve the immune balance of sepsis and regulate the release of inflammatory factors. According to our previous research results, using miR-126 can intervene Th17 and Treg through apoptosis pathway. miR126 plays an important role in immune mediation, we envisage using miR126 to regulate immunity

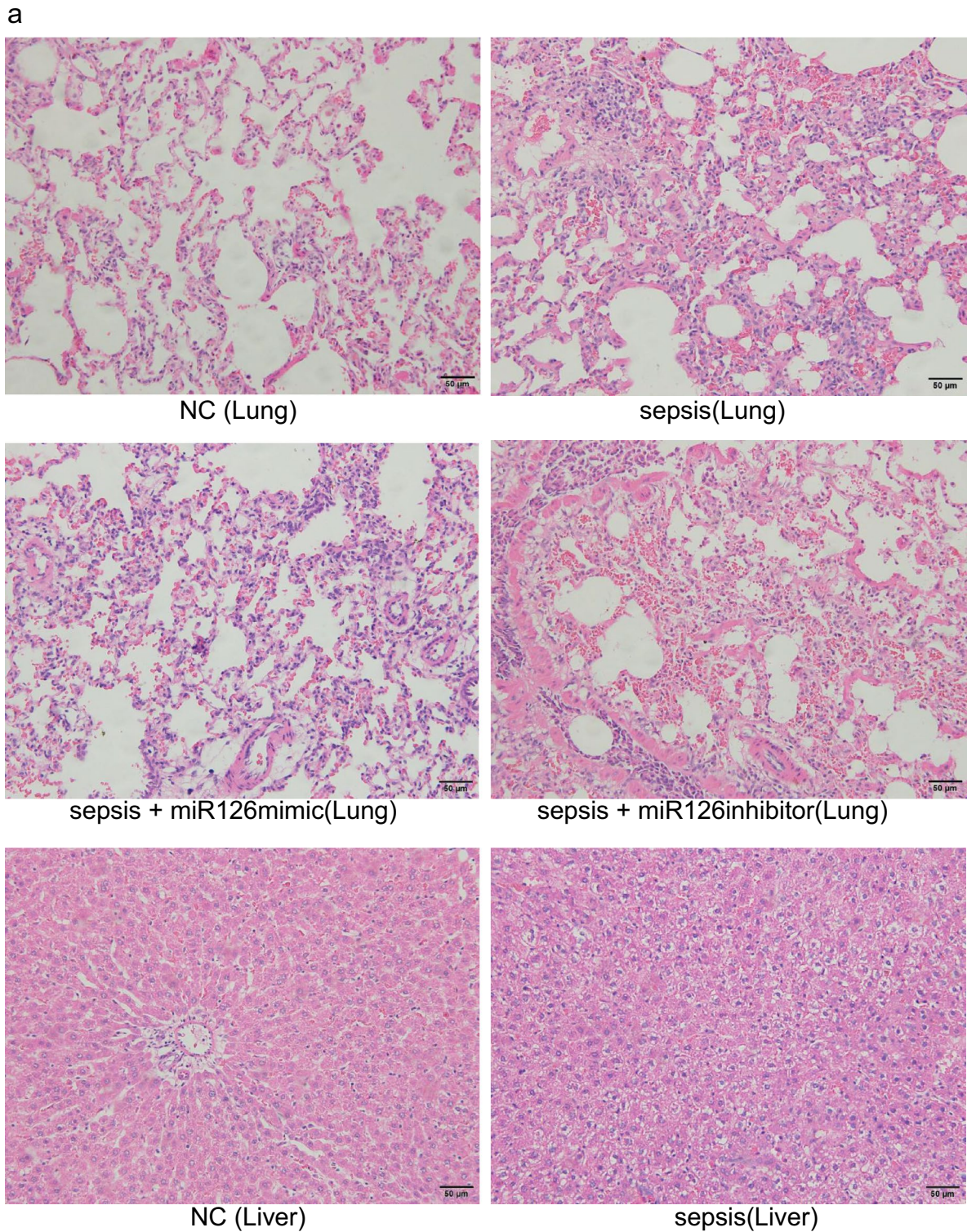
**Fig. 3** The changes of inflammatory factors, Th17 and Treg subsets and the content of miR-126 after transfection with miR-126 mimic and inhibitor in lymphocytes of septic rats. **a)** The expression of miR-126 in lymphocytes of septic rats after transfection of miR-126. **b, c)** The differentiation of Th17 and Treg subsets after miR-126 transfection in septic rats. **d)** The expression of inflammatory factors after transfection with miR-126 mimic and inhibitor in tissue and peripheral blood of septic rats.  $n=8$ , \* $p<0.05$ , \*\* $p<0.01$ . NC normal control

in sepsis, especially the immune function of lymphocytes. Some scholars found that miR-126 can regulate the peripheral induction of CD4 + CD25 + regulatory T cells through PI3K/Akt, suggesting that miR-126 plays an important regulatory role in immune response [25]. It has been found that miR126 increased the expression of CD11a and CD70 in the study of rheumatoid arthritis, mainly by inhibiting the level of dnmit11 protein and inducing gene promoter methylation [26]. Similar conclusions have been reached in the study of systemic lupus erythematosus[27]. A study has shown that exogenous miR-126 and other miRNAs can inhibit the pro-inflammatory cytokines from macrophages stimulated by lipopolysaccharide in MODS caused by sepsis [28]. This is similar to our previous study, so we transfected T lymphocytes with miR-126, and the results showed that TNF- $\alpha$ , IL-6 and IL-17 changed in plasma, which was consistent with the infiltration of inflammatory factors in organs, and the expression of inflammatory factors in plasma was positively correlated with the degree of organ damage. The above results are also consistent with Lin's study, that is, miR-126 is positively correlated with TNF- $\alpha$ ,IL-6 and IL-8 [29].

In sepsis with acute lung injury, animal experiments show that miR-126 can promote Raf / ERK signaling pathway and improve endothelial cell function, suggesting that miR-126 may be a treatment strategy for ALI / ARDS [30]. Clinical studies have shown that the combined expression of miR-126 and other miRNAs can effectively predict ARDS, and among these circulating peripheral blood microRNAs, miR-126 has the most potential to predict 28-day mortality, which is a useful biological indicator [31]. Huang used gene chip technology to analyze the miRNAs, and found that the miR-126 was decreased in ARDS[32], which is consistent with our research. After overexpression of miR-126, the acute lung injury in septic rats was improved, and the infiltration of inflammatory cells in lung tissue decreased accordingly. Studies on liver dysfunction in sepsis have found that miR-126 has a positive regulatory effect in the proliferation and differentiation of hepatocytes by inhibiting the expression of SRY box 9, suggesting that miR-126 has a potential role as a drug target for nucleic acid therapy of liver failure [33]. One study has demonstrated that regulating the miR-126 in sepsis can increase plasma IL-10, inhibit pulmonary vascular leakage, reduce liver and kidney injury, and improve

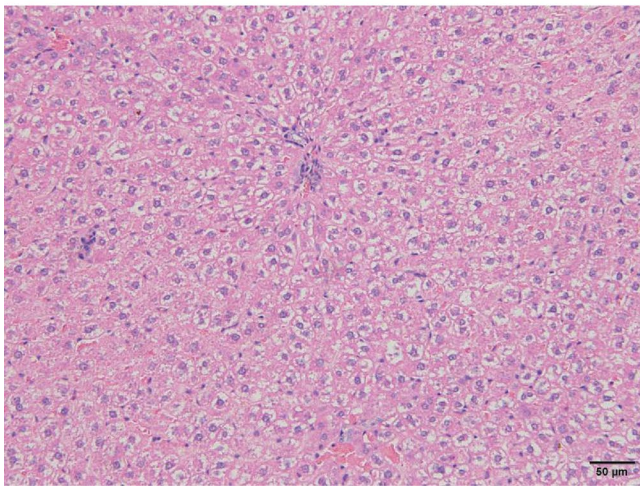




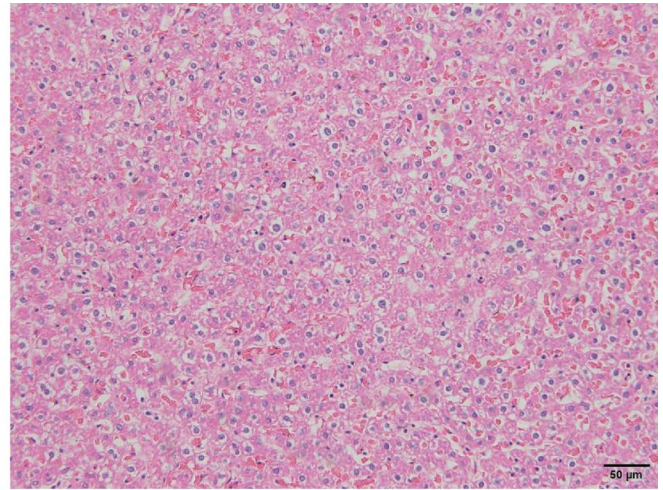


**Fig. 4** The changes of pathological damage and prognosis of organs in septic rats after transfection of miR-126 mimic and inhibitor. **a** After transfection of miR-126 mimic and inhibitor in septic rats, the pathological changes of lung, liver and kidney tissue. **b** After trans-

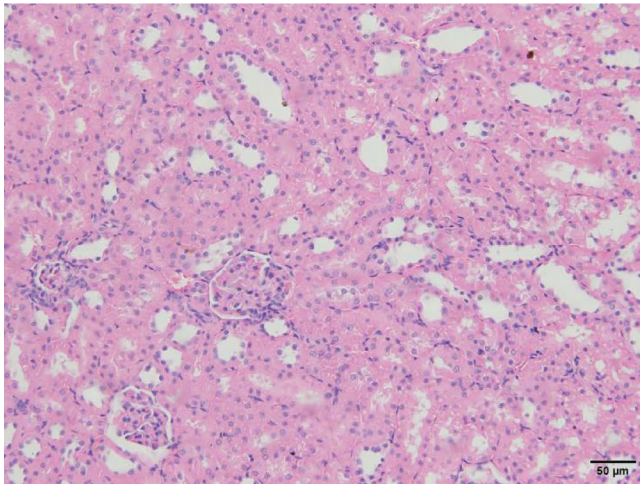
fection of miR-126 mimic and inhibitor in septic rats, the pathological scores of lung, liver and kidney tissue. **c** The survival rate of sepsis rats was compared after transfection of miR-126 mimic and inhibitor. *NC* normal control



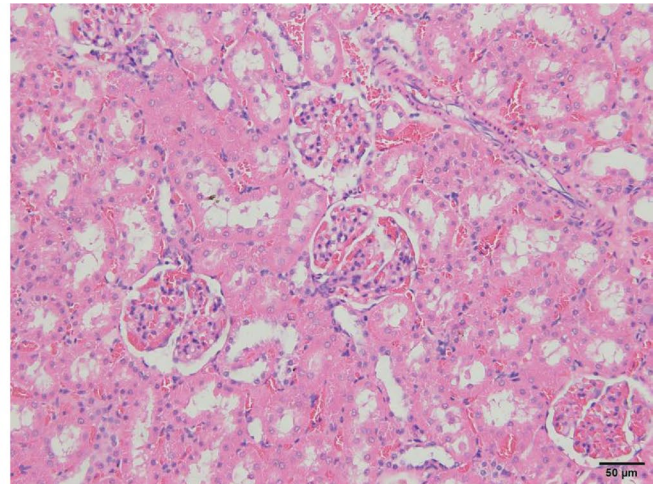
sepsis + miR126mimic(Liver)



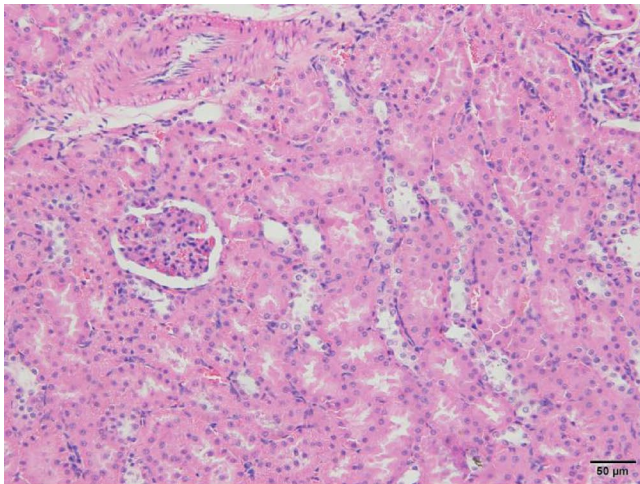
sepsis + miR126inhibitor(Liver)



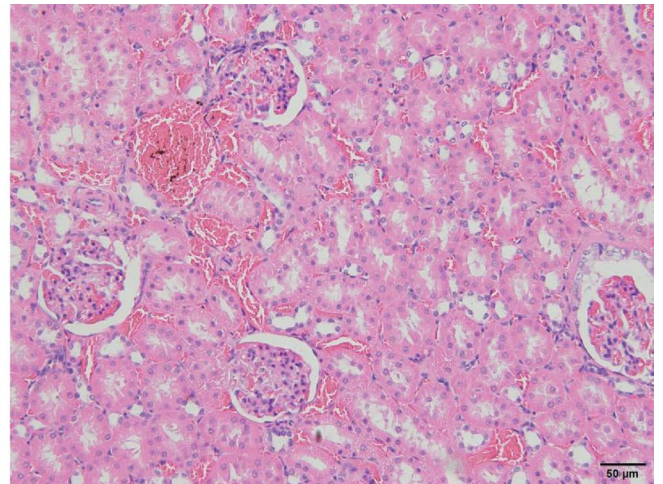
NC(Kidney)



sepsis(Kidney)



sepsis + miR126mimic(Kidney)



sepsis + miR126inhibitor(Kidney)

Fig. 4 (continued)

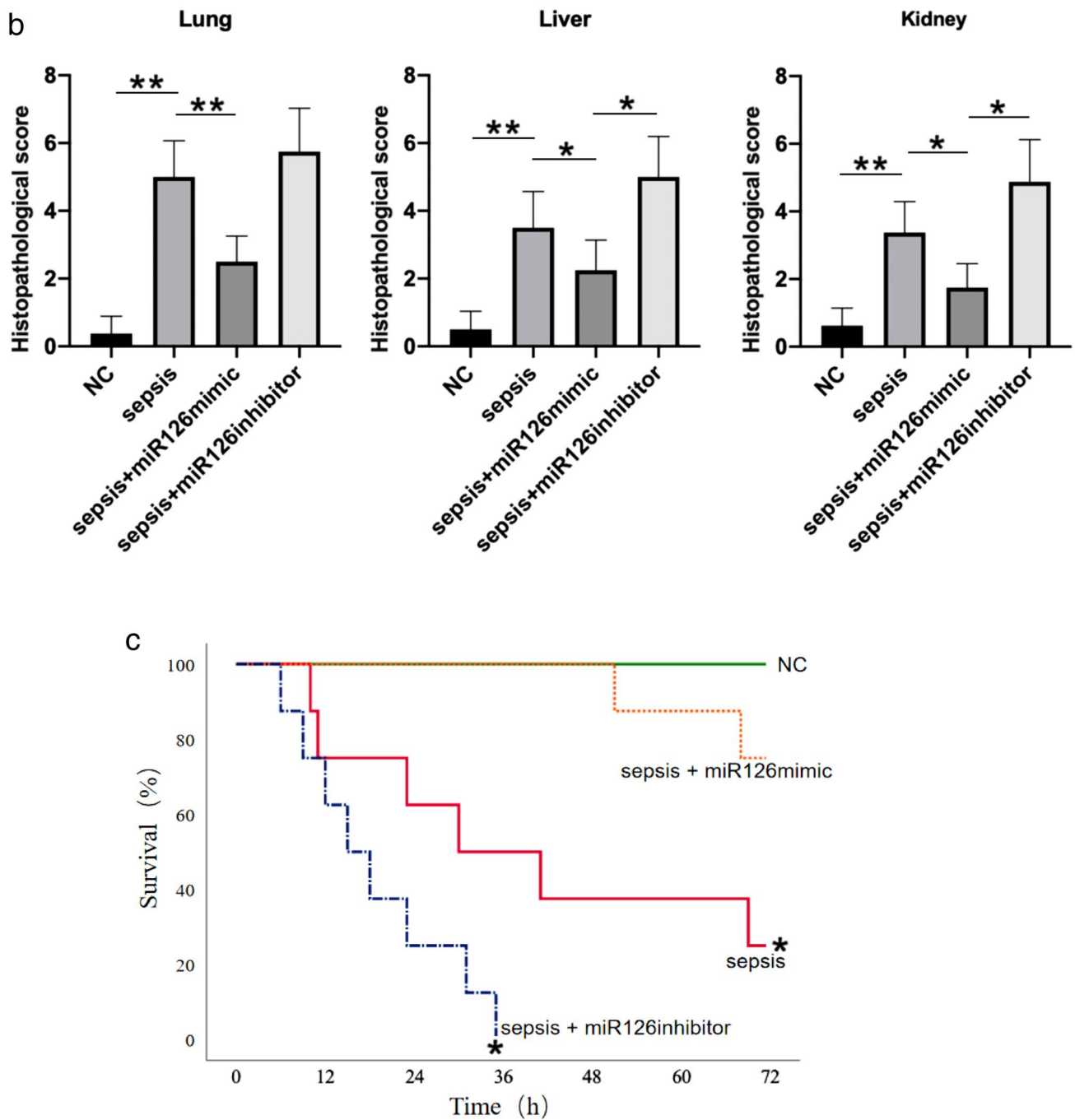
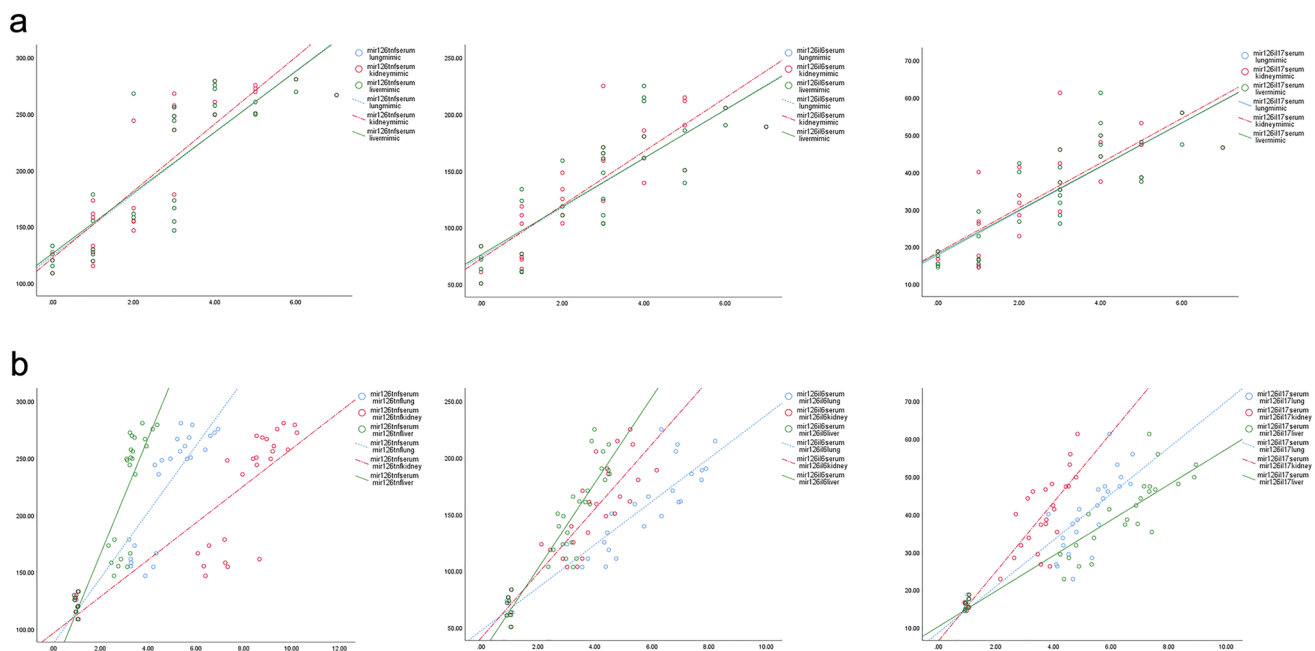


Fig. 4 (continued)

the survival rate of mice [34]. This is similar to the results of our intervention with miR-126. In the miR-126 mimic group, the pathological score of liver injury in sepsis was significantly lower than that in normal rats. Studies have also shown that the miR-126 is decreased in acute kidney injury of sepsis, and overexpression of miR-126 is conducive to maintaining microvascular integrity, reducing kidney injury and accelerating kidney recovery [35]. In terms of

chronic renal insufficiency, the expression of miR-126 in CKD patients is lower than that in normal people, and the degree of reduction is related to the low survival rate of patients [36]. In the damage mechanism of CKD, miR-126 may be involved in renal vascular inflammation response [37]. From the above studies, we can know that the expression of miR-126 is decreased in both acute kidney injury and chronic renal insufficiency. This is also observed in our



**Fig. 5** Correlation analysis of inflammatory factors and pathological damage of organs and infiltration of TNF- $\alpha$ , IL-6 and IL-17 in tissues after transfection of miR-126 mimic and inhibitor in septic rats. **a** Correlation analysis of serum inflammatory factors and pathologi-

cal scores of lung, liver and kidney. **b** Correlation analysis of serum inflammatory factors and the infiltration of inflammatory factors in lung, liver and kidney

study and this decline is associated with the aggravation of organ damage. In addition, we found that the renal function in patients with overexpression of miR-126 was less than that in sepsis.

In the study of sepsis prognosis, one study has shown that the delivery of miR-126 in animals can significantly improve the survival rate, maintain vascular integrity and regulate the production of cytokines [38]. Overexpression of miR-126 can protect podocyte cells from sepsis through EGFL6/dkc1 signaling pathway [39]. MiR-126 can make adipose derived mesenchymal stem cells inhibit histone mediated pulmonary hemorrhage and pulmonary edema, reduce vascular permeability, and significantly improve the survival rate of mice [40]. Our study also proved that miR-126 may improve the prognosis of septic rats, while inhibition of miR-126 can reduce the survival rate of rats. Combined with our previous studies, we consider that miR-126 changes the differentiation of Th17/Treg through apoptosis, leading to changes in the release of inflammatory factors, and ultimately changes the infiltration of inflammatory factors in tissues and organs. Relevant study has also confirmed our view that the overexpression of miR-126 can inhibit the immune response of septic mice through Akt/Rac1, reduce the inflammatory factors and improve the mortality [39].

In conclusion, multiple organ dysfunction is the main cause of death in sepsis, so improving multiple organ dysfunction is of great significance to improve treatment

success rate of patients with sepsis. Through the study, we found that the intervention of miR-126 can play a regulatory role on Th17/Treg, change the inflammatory factors in peripheral blood and tissues, thus affecting the prognosis of septic rats and the degree of organ damage. However, there are still some deficiencies in this study. The regulation of immune function and tissue damage in sepsis are not only related to our previous studies on apoptosis, but also related to autophagy. Next, we will further study the role and mechanism of miR-126 and other miRNAs in sepsis.

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## Declarations

**Conflict of interest** None.

**Ethical approval** This research was approved by the animal ethics committee of Bengbu Medical College (No. 2018074). All applicable international, national and institutional guidelines for the care and use of animals were followed.

**Informed consent** None.

**Consent to participate** All authors confirmed their participation in this study.

**Consent to publish** All authors have agreed to publish this manuscript.

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