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miRNA-1246 suppresses acute lung injury-induced inflammation and apoptosis via the NF-κB and Wnt/β-catenin signal pathways

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ABSTRACT

Acute lung injury (ALI) is the common and complicated inflammatory lung disease. MicroRNAs (miRNA) have emerged as novel gene regulatory molecules which play a crucial role in multiple complicated diseases, including ALI. In this study, we aims to identify potential regulatory functions of miRNA-1246 in lipopolysaccharide (LPS)-induced ALI. In ALI mice, miRNA-1246 expression is effectively up-regulated, compared with the control group. miRNA-1246 overexpression effectively increases inflammation and apoptosis of *in vitro* ALI model. In contrast, miRNA-1246 knockdown effectively inhibits inflammation and cell apoptosis *in vitro* ALI model. Furthermore, up-regulation of miRNA-1246 significantly induces nuclear factor-kappa B (NF-κB) protein expression, and suppresses Wnt and β-catenin protein expression *in vitro* ALI model. Following the inhibition of NF-κB or Wnt/β-catenin signal using inhibitors, miRNA-1246 shows no significant effects on ALI-induced inflammation and apoptosis. Taken together, miRNA-1246 mediates ALI-induced lung inflammation and apoptosis via the NF-κB activation and Wnt/β-catenin suppression.

1. Introduction

Acute lung injury (ALI) occurs in disease process of severe infection, shock, trauma and burn injury [1]. Alveolar epithelial cell and pulmonary capillary endothelial cell injuries will increase alveolar capillary permeability [2]. Moreover, the protein-containing edema fluid aggregates in the alveolar cavity, which induces diffuse pulmonary edema [3]. ALI is characterized by the clinical syndromes of progressive hypoxemia and respiratory distress. Meanwhile, it belongs to the early stage of acute respiratory distress syndrome (ARDS) in terms of its course of disease [1].

Evidences have suggested that microRNA (miRNA) expression is dynamic, which reflects changes in extracellular and intracellular environment and signal [4]. Though miRNA-induced changes in related gene expression are generally moderate [5], such results may affect the subsequent expression of a large amount of genes, thereby influencing multiple biological processes [6,7]. Inflammatory factor release-induced inflammatory response in lung tissue is one of the causes of increased ALI alveolar capillary permeability and disturbance of alveolar fluid clearance [8]. MiRNA expression changes are associated with immune response, inflammatory signaling pathway and the pathogenesis of inflammatory lung disease including ALI [9]. A recent review has discussed characteristics of miRNAs and the regulatory role of miRNAs in the pathogenesis of ALI/ARDS, and found that miRNAs could control inflammatory target gene expression in ALI/ARDS and a small change in miRNA expression could result in marked phenotypic consequences [10]. Furthermore, miRNA can be detected in numerous specimens such as tissue, blood or other fluid. miRNA is quite stable and is not affected by error in sample treatment [11]. Therefore, miRNAs are promising to be served as the novel therapeutic targets.

NF-κB is an important transcription factor, which plays a vital role in regulating the production of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6. NF-κB can translocate from cytoplasm to cell nucleus under the stimulation of LPS [12]. Therefore, it can initiate transcription of genes such as pro-inflammatory cytokine, inflammatory mediators and chemokines. Consequently, intervention targeting the NF-κB active site is imperative [13].The Wnt/β-catenin pathway is the canonical Wnt signaling pathway, the signal transduction process of which is as follows· Wnt signal protein binds with the transmembrane receptor, thus activating the Wnt signaling pathway [14,15]. Later, shock protein and GSK-β binding protein are activated in succession [15]. The latter can identify and inhibit the phosphorylation activity of GSK-3β, so that it can not phosphorylate β-catenin [16]. As a result, β-catenin can not be identified by the ubiquitin ligase and degraded by protease complex. The number of β-catenin in cell nucleus is surging, and transcription of target gene is initiated eventually [15]. Recent

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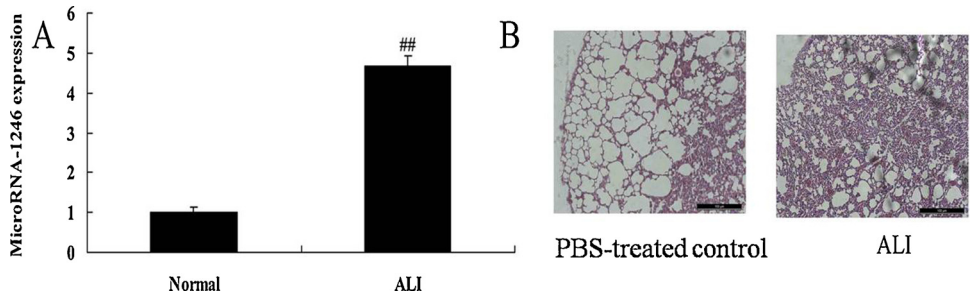

Fig. 1. miRNA-1246 expression in ALI mice.
MiRNA-1246 expression (A) and H&E staining (B) are detectedin normal control group and ALI model group. ##p < 0.01 versus normal control group.

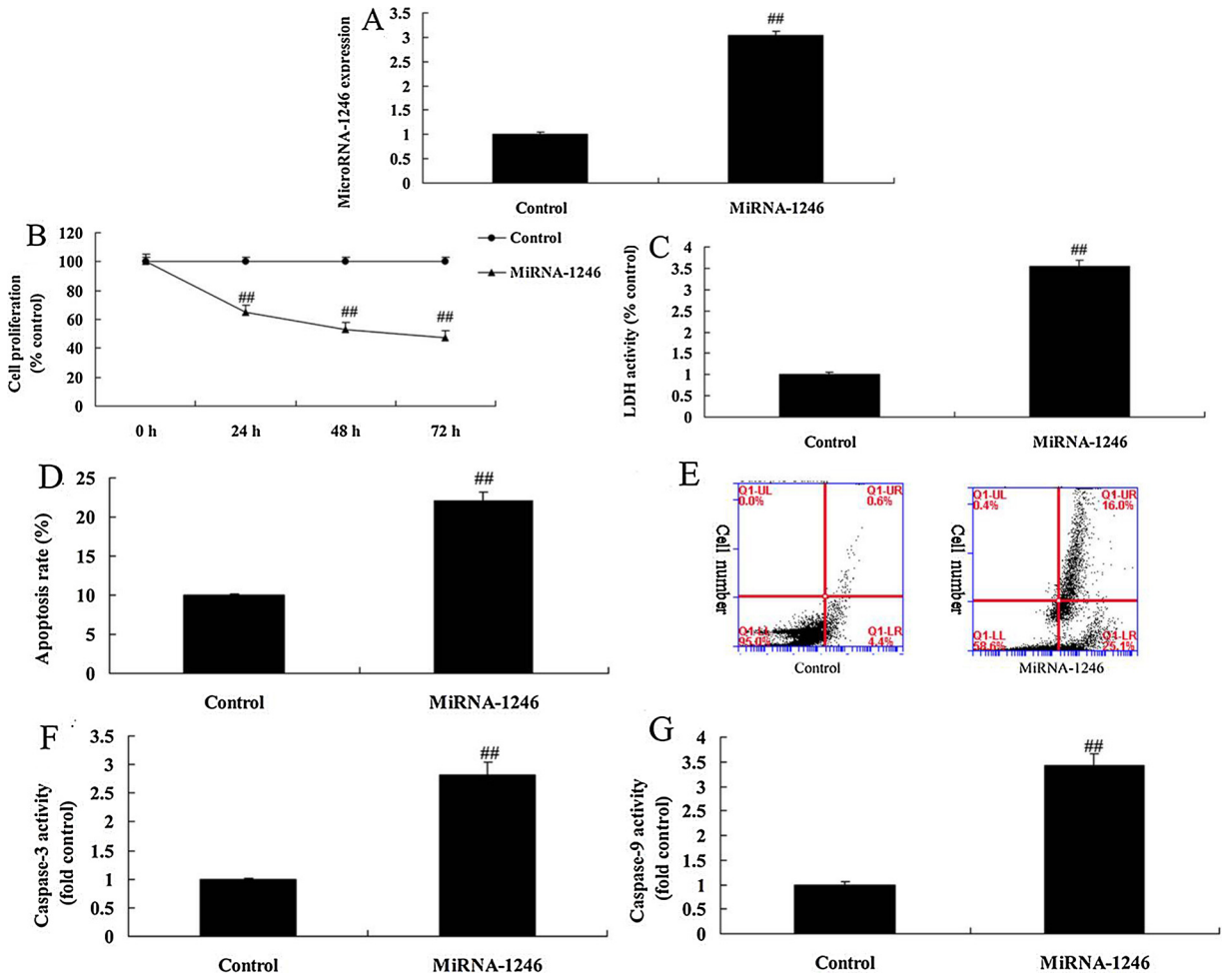

Fig. 2. miRNA-1246 effectively increases cell apoptosis *in* ALI cell model. miRNA-1246 expression (A), cell proliferation (B) and LDH activity (C), the apoptosis rate (D and E), and caspase-3/9 activity (F and G) are measured In the control group and miRNA-1246 overexpression group.
##p < 0.01 versus the control group.

research has indicated that the abnormal activation of the Wnt signaling pathway plays a key role in the fibrosis genesis and development of organs such as liver, kidney, lung, heart and skin [16]. Researchers in the studies of human tumors found that a variety of miRNAs can regulate the function of the Wnt/β-catenin signaling pathway. One study suggested that miR-200a directly targets β-catenin mRNA, which contains a functionally conserved miR-200a-binding site in its 3′UTR and suppresses β-catenin/Wnt signaling, which is implicated in tumorigenesis in human colon cancer, hepatocellular carcinoma, melanoma, ovarian cancer and prostate cancer [17]. Chai et al. [18] showed that Octamer 4/miRNA-1246 signaling is activated in CD133 liver cancer stem cells (CSCs) through Wnt/β-catenin activation and the pathway is related with caspase-mediated cell apoptosis [18].

In the present study, we hypothesize that miRNA-1246 may mediate ALI-induced lung inflammation and apoptosis via the NF-κB and Wnt/β-catenin signal pathways. Further, we aim to identify potential regulators of miRNA-1246 on ALI and to explore their specific roles in lipopolysaccharide (LPS)-induced ALI.

2. Materials and methods

2.1. Protocol

A total of 20 C57BL/6 mice were randomly divided into phosphate