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Original Article

Argonaute proteins in cardiac tissue contribute to the heart injury during viral myocarditis

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ABSTRACT

MicroRNAs (miRNAs) are a group of short, noncoding, regulatory RNA molecules the dysregulation of which contributes to the pathogenesis of myocarditis. Argonaute proteins are essential components of miRNA-induced silencing complex and play important roles during miRNA biogenesis and function. However, the expression pattern of four AGO family members has not yet been detected in the coxsackievirus B3 (CVB3)-induced myocarditis tissue samples. In this study, we detected the expression of four AGOs in the CVB3-infected mouse heart tissues and found that AGO1 and AGO3 up-regulated significantly at 4 and 8 h after CVB3 infection. Further in vitro research indicated that up-regulated AGO1 and AGO3 are related to the down-regulated TNFAIP3, which is a negative regulator of NF-κB pathway. Subsequently, we confirmed that TNFAIP3 is a direct target of miR-19a/b, and during CVB3 infection, the expression of miR-19a/b and miR-125a/b is not significantly changed. TNFAIP3 level is mainly reduced by up-regulated AGO1 and AGO3. This research sheds light on the relationship between overexpressed AGO proteins and CVB3-induced myocarditis, and this provides potential therapeutic target for viral myocarditis.

1. Introduction

Myocarditis is defined as an inflammation of heart muscle that most often induced by enteroviruses infection and from 4% to 20% of sudden cardiovascular deaths among young adults, the military and athletes, are due to myocarditis [1]. Although most cases of suspected myocarditis are not linked to a specific cause, viral infections like coxsackievirus B3 (CVB3) are the most commonly identified cause of myocarditis [2,3].

The pathogenesis of VM is based on an adverse immune response evoked by infection of the cardiac muscle by cardiotropic viruses, which leads to viral elimination as well as cardiac myocyte destruction, reparative fibrosis, and heart failure. The lack of effective therapies to treat myocarditis mandates a better understanding of the basic molecular mechanisms that govern the adequate and autodestructive inflammatory signaling pathways within the immune system [3].

MicroRNAs (miRNAs) are a group of short, noncoding RNA molecules that regulate gene expression through targeting the 3′untranslated region (UTR) of mRNA sequences. Argonaute proteins are essential components of miRNA-induced silencing complex and play important roles during miRNA biogenesis and function. There are four argonaute proteins (AGO1–AGO4) in mammalian cells and emerging evidences indicated that all of the four AGOs contribute to the process of inhibition of protein coding genes that is reduced by miRNA.

Recently, important roles of miRNA during the pathogenesis of myocarditis have been partially examined. However, the expression of AGO1–AGO4 is still not being checked in myocardial tissues of patients or mice with myocarditis. In this study, we detected the expression of AGO1–AGO4 in heart tissues of myocarditis mouse induced by CVB3 and found a disturbed distribution of AGO1–AGO4. Further in vitro study indicated that altered AGO1–AGO4 expression related to enhanced immune response by repressing NF-κB pathway inhibitors.

2. Materials and methods

2.1. Mice

All experiments were carried out in accordance with the guiding principles for research involving animals and human beings. Ethical approval was obtained from the Animal Ethics Committee of Lanzhou University Second Hospital before the start of the study. Specific pathogen-free male BALB/c mice (6 weeks old) were housed under pathogen-free

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conditions in an animal house and fed normal mouse chow and given tap water ad libitum.

2.2. CVB3 virus and mice infection

CVB3 (Nancy strain), which was bountifully provided by the Wuhan University, was propagated in Hela cell monolayers and then stored at −80°C. The supernatant from infected cell cultures was collected, and viral titers were determined in 96-well plates by plaque assay.

Six-week-old male mice were injected intraperitoneally with 10^4 plaque-forming units of CVB3 that was diluted in 0.1 ml PBS. Mice were observed every day for the development of clinical morbidity and mortality until day 8. Mice administered intraperitoneal injection with 0.1 ml PBS were taken as control group and according to the time point postinjection. The myocarditis was confirmed by histologic evaluation. The heart tissue samples were collected at days 0, 4, and 8 from three mice of each time point and each group.

2.3. RNA extraction and qRT-PCR

Total RNA was extracted from tissue samples or cells by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was used to determine the relative expression level of candidate genes. The expression of β-actin was used for normalization. Each sample in each group was measured in triplicate and the experiment was repeated at least three times.

The expression of miR-125a/b and miR-19a/b was detected by TaqMan miRNA RT-Real Time PCR. Single-stranded cDNA was synthesized by using TaqMan MicroRNA Reverse Transcription Kit (Applied

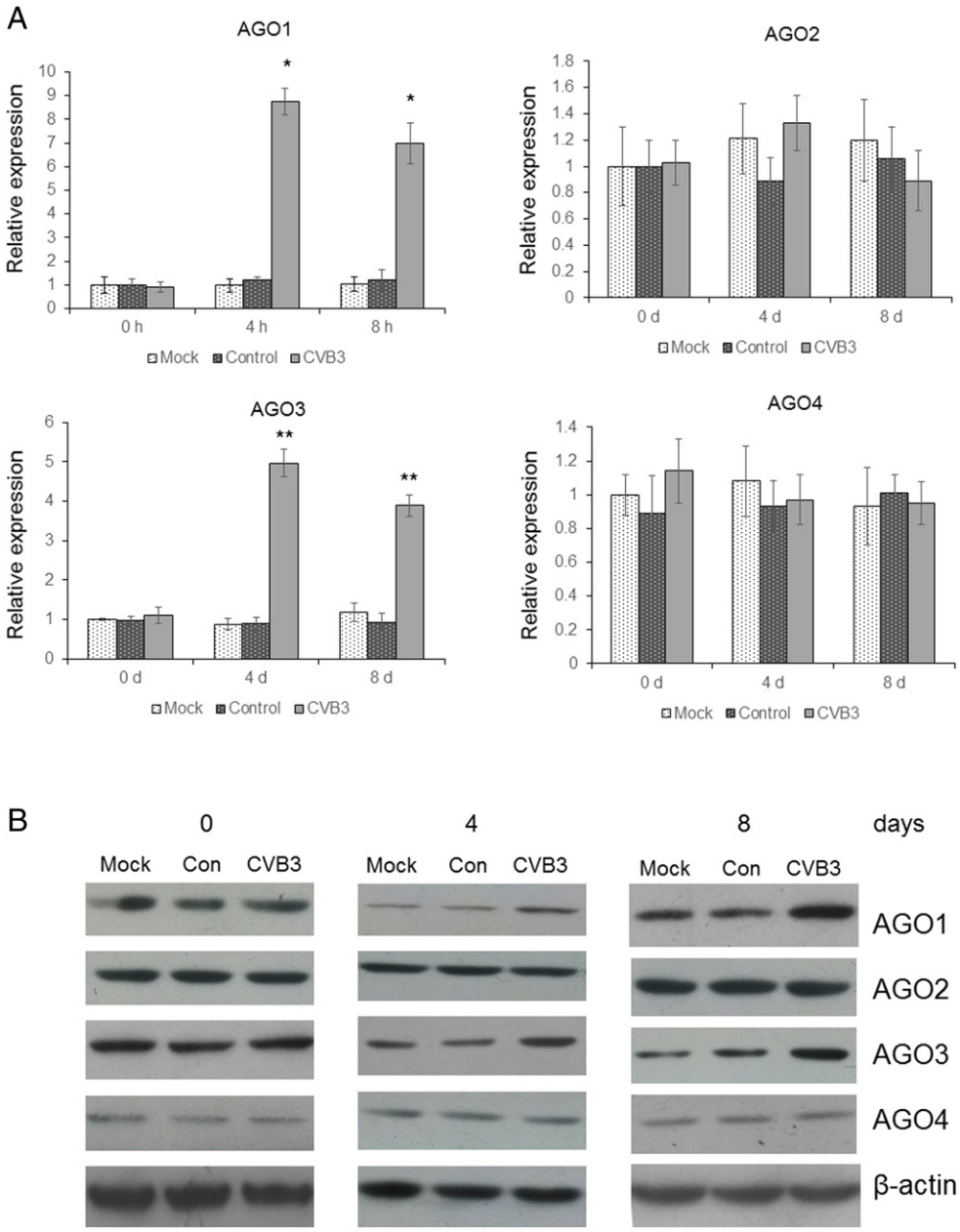

Fig. 1. AGO1 and AGO3 are up-regulated in the heart tissue of CVB3-infected mice. Six-week-old male mice were injected intraperitoneally with 10^4 plaque-forming units of CVB3 that was diluted in 0.1 ml PBS. Mice were observed every day for the development of clinical morbidity and mortality until day 8. Mice administered intraperitoneal injection with 0.1 ml PBS or without any treatment were taken as control group and according to the time point postinjection. The heart tissue samples were collected at days 0, 4, and 8 from six mice of each time point and each group. The expression of AGO1–AGO4 was detected by qRT-PCR (A) and western blot (B). The results were analyzed by Student's *t* test and $P<.05$ was considered statistically significant. $^*P<.05$.