OBJECTIVES

Post-ischemic inflammation is an essential step in the progression of myocardial infarction (MI). Damage-associated molecular patterns (DAMPs) are implicated in the activation of infiltrating immune cells and triggering release of inflammatory cytokines. MicroRNA-21 (miR-21) has been shown to inhibit apoptosis of cardiomyocytes and promote cardiac fibrosis. However, the role of miR-21 in post-ischemic inflammation remains obscure. Our aim is to determine whether miR-21 regulates DAMPs-triggered inflammation after MI in mice and investigate the underlying signal mechanism.

METHODS

We constructed the GFP adenovirus vector expressing miR-21 (GFP-Ad-miR-21) and control adenovirus vector (GFP-Ad). For in vivo experiment, myocardial infarction of mice was induced by left coronary ligation and then the GFP-Ad-miR-21 or GFP-Ad was directly injected into the remote region of infarct heart. Mice were divided into four groups: MI-Ad-miR-21, MI-Ad, sham-Ad-miR-21, sham-Ad. The LVEF, LVFS, LVESD and LVEDD were detected by echocardiography 60 days after MI induction and the myocardial infarct size was analyzed by TTC staining. The inflammatory cytokines were evaluated by Q-PCR, ELISA and IHC. Meanwhile, DAMPs-induced inflammatory response of macrophage was simulated by stimulation with recombinant mouse HSPr60 (rmHSPr60) or recombinant mouse HMGB1 (rmHMGB1) after transfection with miR-21 mimics. The expressions of inflammatory cytokines and the activation of MAPK and NF-κB signal in macrophage were determined, respectively. In mechanism, we analyzed the target genes using TargetScan 6.2 and verify the interaction between miR-21 and targeting gene with luciferase reporter assay.

RESULTS

In MI-Ad-miR-21, LVEF and LVFS were significantly decreased, and LVESD, LVEDD and myocardial infarct size were apparently increased. In heart tissue of MI-Ad-miR-21, the expression of IL-1β, IL-6, TNF-α and NF-κB were higher than that in MI-MI. Overexpression of miR-21 mimics notably increased the production of IL-1β, IL-6, IL-12 and TNF-α in mouse macrophages stimulated with rHSP60 or rHMGB1. Moreover, the phosphorylated levels of ERK, JNK, p38 and IKK-β were significantly increased when overexpression of miR-21 mimics in the macrophages. Furthermore, we found that miR-21 mimic markedly decreased the luciferase activity of wild type STK40 luciferase (STK40-Luc). Consistently, miR-21 inhibitor increased the luciferase activity of wild type STK40-Luc. In addition, the expression of STK40 was found to be reduced after transfection with miR-21 mimics in macrophages.

CONCLUSIONS

These data show that miR-21 up-regulates inflammatory response after MI by enhancing the activation of MAPK and NF-κB signaling pathway. Mir-21 inhibits the protein and mRNA expression levels of the STK40 in mouse macrophages, together with previous study that STK40 is the negative regulator of MAPK and NF-κB pathway, these data indicate that miR-21 promote MI-induced inflammatory response by regulating the target gene STK40.