GW23-e1176 **THE ROLES OF PRESENILIN-2 GENE IN THE APOPTOSIS OF MYOCARDIAL CELL**

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Objectives Presenilin (PS) gene is a novel gene family of causative gene related with familial Alzheimer's disease. They have been detected not only in the brain, but also in some peripheral tissues including the heart. The main focus on the PS gene family was in central nervous system. RNA interference (RNAi) is a kind of technology including small interfering RNA (siRNA) and microRNA (miRNA). miRNAs are small noncoding RNAs that participate in regulating gene expression at post-transcriptional level. They play important roles in the cell growth, differentiation, proliferation, metabolism, and apoptosis. It has been shown that miRNAs participate in the regulation of a diverse spectrum of cardiac functions with developmental, pathophysiological and clinical implications. Research found that PS2 gene mutations are associated with DCM, and implicated novel mechanisms of myocardial disease. It has been believed that PS2 gene regulate the cell apoptosis via mechanism known as Ca^{2+} induced-Ca^{2+} release in nervous system. However, PS2 gene function and its regulation in the myocardial cell apoptosis has not been established. To construct the specific miRNA expression vectors targeting PS2 gene, and to investigate the effects of PS2 gene on the apoptosis of rat H9c2 cells using RNAi.

Methods

1. Four miRNAs targeting the PS2 gene were synthesised and inserted into the pcDNATM6.2-GW/EmGFP miR vectors.
2. The recombinant plasmids were identified and transiently transfected into rat H9c2 cells via lipofectamine 2000. After 48 h of transfection, the transfection efficiency was monitored by inverted fluorescence microscopy and flow cytometry (FCM). The efficacy of RNAi at the mRNA and protein levels were assessed by real-time fluorescent quantitive PCR (FQ-PCR) and Western bolt, respectively.
3. The myocardial cell apoptosis models of rats were established, H9c2 cells was treated with 100 μmol/l Hydrogen peroxide (H_2O_2) for 12 h, and the apoptosis of H9c2 cells treated by RNAi, compared with those containing control vectors or untreated, were analysed using FCM and Cell Counting Kit-8 (CCK-8) assay.

Results

1. The recombinant miRNA expression vectors, which target PS2 gene was successful constructed.
2. Compared with the control group, the expressions of PS2 mRNA in H9c2 miRNA-trasnfected cells were not significantly down-regulated ($p>0.05$). However, Western blot demonstrated that the H9c2 cells transected with PS2 miRNA plasmid resulted in a dramatic down-regulation of PS2 protein ($p<0.001$).
3. Myocardial H9c2 cells cultivated in vitro were treated with H_2O_2 (50, 100, 200 μmol/l) in control and experimental groups respectively. Apoptosis was determined by AO-PI double fluorescent staining assay, the results showed that H_2O_2 could damage H9c2 cells in dose and time dependent way, and induce apoptosis with obviously morphological changes in exposed groups at 12 h, 100 μmol/l level of H_2O_2 ($p<0.05$). FCM showed an increased cell apoptosis rate in PS2 miRNA group compared with that in the positive and negative control group ($p<0.05$). CCK-8 assay revealed that the proliferation inhibitory rate was higher in PS2 miRNA group than in control group ($p<0.001$).

Conclusions The expression plasmids carrying miRNA targeting PS2 gene have been successfully constructed. Transfection of these plamsids can efficiently inhibit PS2 gene expression in protein level of the rat H9c2 cells. PS2 gene may inhibit the hydrogen peroxide-induced apoptosis of rat H9c2 cells.