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THE EFFECT OF ROCK1 AND ROCK2 SILENCING BY SHRNA ON APOPTOSIS INDUCED BY HYPOXIA IN RAT CARDIOMYOCYTE

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Objectives It is well known that the apoptosis of cardiomyocyte involves the development of some cardiovascular diseases, such as heart failure, coronary heart disease and so on. Down-regulation of ROCK1 (Rho—associated coiled-coil protein kinase-1) and ROCK2 could inhibit apoptosis of a variety of cells. Therefore, shRNAs targeting ROCK1 and ROCK2 were transfected into rat cardiomyocytes to inhibit the expression of ROCK1 and ROCK2. The effect of ROCK1 and ROCK2 on apoptosis induced by hypoxia was studied in rat cardiomyocyte.

Methods

1. Rat cardiomyocytes were cultured primarily and identified by using antibody targeting α-actin of striated muscle.
2. Three recombinant plasmids of ROCK1-shRNA and ROCK2-shRNA were constructed respectively and identified.
3. ROCK1-shRNA and ROCK2-shRNA were transfected transiently into cells by liposome. After 48 h, the protein of ROCK1 and ROCK2 was isolated and detected through Western

Blotting. The shRNAs with the best silencing efficiency were selected from these shRNAs.

4. ROCK1-shRNA and ROCK2-shRNA with the best silencing efficiency were transfected into cardiomyocytes. After 48 h, these cells were subjected to hypoxia for 6 h. The experiments were divided into five groups: Blank Control Group, Hypoxia Group, Hypoxia +Negative Control shRNA Group, Hypoxia+ROCK1-shRNA Group, Hypoxia+ROCK2-shRNA Group. The expression of green fluorescent protein was observed through fluorescence microscopy.
5. Followed by treatment with transfection and hypoxia, Western Blotting was used to determine the expression of Caspase3、p-PI3K and PI3K, cardiomyocyte beat frequency and rhythm was assessed using microscopy, the content of lactate dehydrogenase (LDH) in cell culture fluid was detected by automatic biochemical analyser, cell survival rate was determined with MTS, cell apoptosis rate was assessed by using flow cytometry.

Results

1. It was confirmed that rat cardiomyocytes were cultured successfully.
2. Western blotting results showed that the transfection of ROCK1-shRNA and ROCK2-shRNA inhibited the expression of ROCK1 and ROCK2 effectively, ROCK1-shRNA1 and ROCK2-shRNA2 have the best silencing efficiency.
3. By fluorescence microscopy, green fluorescences were observed in the cells transfected with shRNA, indicating that these recombinant plasmids were transfected successfully.
4. Hypoxia decreased the beat frequency and extent of cardiomyocyte, made rhythm disorder, while the transfection of ROCK1-shRNA and ROCK2-shRNA reduced the effects caused by hypoxia.
5. Automatic biochemical analyser showed that hypoxia increased the content of LDH, while the transfection of ROCK1-shRNA and ROCK2-shRNA reduced the effects caused by hypoxia.
6. MTS results showed that hypoxia decreased cell survival rate, while the transfection of ROCK1-shRNA and ROCK2-shRNA reduced the effects caused by hypoxia.
7. Flow cytometry results showed that hypoxia increased cell apoptosis rate, while the transfection of ROCK1-shRNA and ROCK2-shRNA reduced the effects caused by hypoxia.
8. Western blotting results showed that hypoxia increased the expression of Caspase3 and decreased the expression of p-PI3K, while the transfection of ROCK1-shRNA and ROCK2-shRNA reduced the effects caused by hypoxia. The expression of PI3K wasn't changed.

Conclusions

1. The expression of ROCK1 and ROCK2 in rat cardiomyocyte can be inhibited effectively by the transfection of ROCK1-shRNA and ROCK2-shRNA.
2. The down-regulation of the expression of ROCK1 and ROCK2 can reduce the effects, caused by hypoxia, that the proliferation of rat cardiomyocyte was attenuated and the apoptosis was enhanced.
3. It is through ROCK1 and ROCK2 that the expression of Caspase3 and p-PI3K is influenced by hypoxia.
4. A new possible method is provided for treatment of some cardiovascular diseases, such as heart failure and coronary heart disease.