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Author: Mrs. Jia Liu
China Astronaut Research and Training Center, China, moondream1020@163.com

Mr. Hongwei Zheng
China Astronaut Research and Training Center, China, 184133007@qq.com
Mrs. Yuan Min
State Key Laboratory of Space Medicine Fundamentals and Application, China Astronaut Research and Training Center, China, y_min5628@yahoo.com.cn
Mrs. Jingyu Wang
State Key Laboratory of Space Medicine Fundamentals and Application, China Astronaut Research and Training Center, China, icandowjy@163.com
Dr. Ming Yuan
1 Space Institute of Southern China(Shenzhen), 2 China Astronaut Research and Training Center, China, yuanming7@hotmail.com

MIRNA SEQUENCING AND BIOINFORMATICS ANALYSIS OF VASCULAR ENDOTHELIAL CELLS TREATED BY OXIDATIVE STRESS UNDER SIMULATED MICROGRAVITY

Abstract

Microgravity or simulated microgravity(SM) can induce cardiovascular dysfunction in which vascular endothelial cells play an important role. Studies showed that oxidative stress was common in vascular tissues under simulated microgravity and was also increased in vascular endothelial cells during spaceflight. miRNAs have important role in the regulation of physiological function in endothelial cells. The purpose of this experiment is to study whether vascular endothelial cells have different miRNAs profile response to oxidative stress under simulated microgravity with miRNA sequencing technology. EA.hy926 cells, a fusion of HUVECs and the lung carcinoma cell line A549, were cultured in clinostat for 72h to simulate the effects of microgravity, then the cells treated by clinorotation were stimulated with H2O2 for 5 hours. miRNA sequencing was performed to screen the differentially expressed miRNAs which were verified by qRT-PCR. The results showed that there were 97 differential miRNAs in the cells treated by clinorotation compared with control cells(Clino vs C group) in which 17 up-regulated and 80 down-regulated, 102 differential miRNAs in H2O2 treated control cells compared with control (C+ H2O2 vs C group) in which 53 up-regulated and 49 down-regulated, 71 differential miRNAs in H2O2 treated clinorotation cells compared with cells only treated by clinorotation(Clino+H2O2 vs Clino group) in which 52 up-regulated and 19 down-regulated, 66 differential miRNAs in H2O2 treated clinorotation cells compared with H2O2 treated control cells (Clino+H2O2 vs C+ H2O2 group) in which 38 up-regulated and 28 down-regulated. qRT-PCR result showed that hsa-miR-210-3p increased in Clino+ H2O2 group compared with C+ H2O2 group, which was consistent with the data from miRNA sequencing. Bioinformatics analysis showed that there were 553 target genes for hsa-miR-210-3p, GO analysis showed that the functions of these target genes were enriched in molecular function(MF) of binding(GO:0005488) and catalytic activity(GO:0003824), KEGG pathway analysis showed that Focal adhesion, PI3K-Akt signaling pathway, ubiquitin mediated proteolysis, MAPK signaling pathway were significantly enriched, and STRING protein interaction network showed that there were 552 protein nodes and 712 edges identified, and SKP1, TCEB2, GTF2B EIF4EBP1 and FOS were the top 5 hubs. From these results, we concluded that oxidative stress under simulated microgravity induced different miRNAs regulatory response in EA.hy926 cells including

hsa-miR-210-3p, and the underlying mechanisms deserve to study in follow-up. (Funded by State Key Laboratory of Space Medicine Fundamentals and Application, China Astronaut Research and Training Center, grant NO. SMFA14B01)