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Effect of AMPK agonist / inhibitor on Nrf2 expression in C2C12 cells

Lin Luo[1],Ying Zhang[2]
1.College of physical education， Guizhou Normal University
2.College of sports human science， Beijing Sport University

Objective In the past few decades, the study of skeletal muscle oxidative stress has been concerned about the increase of free radicals induced by muscle contraction. In recent years, the activation of antioxidant stress signaling pathway has gradually become one of the hot topics in the field of sports medicine. Although current research has confirmed that long-term aerobic training can bring health benefits to the body, the molecular mechanism of its role is still not very clear.Traditionally, AMPK has been regarded as the energy receptor of cells. During exercise, the energy consumption of skeletal muscle doubled, ATP decreased, AMP increased, and the ratio of AMP/ATP increased, thus inducing the activation of AMPK and regulating cell energy metabolism. Recent studies have found that AMPK not only plays an important role in the regulation of energy metabolism, but also plays a role in the body's antioxidant stress response. However, the relationship between AMPK and oxidative stress has been studied only in a small number of cells in non skeletal muscle cells. The results of this few studies show that oxidative stress in AMPK can not depend on the increase of intracellular AMP/ATP ratio, and the independent activation of AMPK, thus reducing the level of intracellular ROS, but the molecular mechanism of its action is not clear. Nrf2 is an important nuclear transcription factor in the body and plays an important role in the body's antioxidant stress response. Whether AMPK can participate in the regulation of Nrf2 mediated antioxidant activity in skeletal muscle has not been reported.In this study, the mouse skeletal muscle C2C12 cells were used in vitro cell experiments. The AMPK pharmacologic activator AICAR and the pharmacological inhibitor Compound C were used to treat the cells respectively. The role of AMPK in the regulation of Nrf2 expression in C2C12 cells and its mechanism were observed.
Methods Cell experiments were performed on C2C12 cells of skeletal muscle of mice, and AMPK activator AICAR and AMPK inhibitor Compound C were used to intervene. The fluorescence intensity of C2C12 cells in each group was qualitatively detected by fluorescence inverted microscope, and the ROS level of C2C12 cells in each group was detected by fluorescence colorimetry. Results the ROS level of each group was significantly higher than that of the control group. RT-PCR assay was used to detect the antioxidant enzyme mRNA level of C2C12 cells in each group. Western Blot assay was used to detect the expression of AMPK alpha, pAMPK alpha, Nrf2, pNrf2 and antioxidant enzyme protein in C2C12 cells of each group.
Results (1) compared with the control group, the pAMPK alpha /AMPK alpha ratio of C2C12 cells in the agonist group increased significantly, the expression of pNrf2 protein in the cells increased significantly, and the expression of NQO1mRNA, HO-1mRNA and GSR mRNA increased significantly, and the cells SOD1, GCLM, NQO1, HO-1, pNrf2, and protein were significantly increased. Low. (2) compared with the control group, the levels of NQO1mRNA, HO-1mRNA, CATmRNA, SOD1mRNA, Gpx-1mRNA and GCLc mRNA in the C2C12 cells of the inhibitor group decreased significantly, and the expression of NQO1 and GCLM proteins in the cells decreased significantly, and the ROS level of the cells increased significantly.
Conclusions (1) the activation of AMPK by AICAR activates the increase of Nrf2 activation in skeletal muscle C2C12 cells, and then increases the expression of mRNA and protein (SOD1, GCLM, NQO1, NQO1, GSR) in the downstream of Nrf2 (NQO1, HO-1, GSR), and significantly reduces the

intracellular level.(2) the inhibition of AMPK by Compound C significantly decreased the mRNA expression of C2C12 cells (NQO1, HO-1, CAT, SOD1, Gpx-1, GCLc) in skeletal muscle, and significantly decreased the expression of protein (NQO1 and GCLc).