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shRNA对大鼠心肌细胞牛磺酸转运体的抑制作用

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Inhibitory Effects of shRNA on Taurine Transporter of Rats Cardiac Myoblasts

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摘要 本试验旨在研究短发夹RNA(shRNA)对大鼠心肌细胞牛磺酸转运体(TauT)的抑制作用。根据已克隆的大鼠心肌*TauT*基因序列设计并构建了3个shRNAs(shRNA1#、shRNA2#、shRNA3#)及1个阴性对照shRNA表达载体,利用脂质体Lipofectamine^{TM} 2000将构建好的重组质粒shRNA1#、shRNA2#、shRNA3#及阴性对照质粒分别转染H9c2细胞,细胞分为对照组(未转染组)、shRNA-Neg组(阴性对照组)和3个转染组(shRNA1#组、shRNA2#组、shRNA3#组),每组3个重复。应用四唑盐(MTT)法检测H9c2细胞增殖情况,实时荧光定量PCR检测*TauT* mRNA表达水平。结果表明,与阴性对照组和未转染组比较,shRNA1#质粒仅在转染细胞后的24 h极显著降低了*TauT* mRNA表达水平($P<0.01$),shRNA2#质粒在转染细胞后的24、48、72 h均极显著降低了*TauT* mRNA表达水平($P<0.01$),shRNA3#质粒则是在转染细胞后的72 h极显著降低了*TauT* mRNA表达水平($P<0.01$);MTT检测结果表明,转染*TauT* shRNA1#、shRNA2#、shRNA3#入H9c2细胞24、48、72 h后,细胞增殖均未受到显著影响($P>0.05$)。上述结果提示,shRNA2#重组质粒能有效抑制*TauT*基因的表达,且短期内未影响H9c2细胞增殖,为研究*TauT*基因及揭示牛磺酸在心肌细胞代谢中的作用奠定基础。

关键词： H9c2细胞 RNA干扰 牛磺酸转运体 细胞增殖

Abstract： This experiment was conducted to investigate the inhibitory effects of short hairpin RNA (shRNA) on taurine transporter (TauT) of rats cardiac myoblasts. Three shRNAs expression vectors (shRNA1#, shRNA2# and shRNA3#) and one negative control shRNA expression vector were constructed to be targeted directly at *TauT* gene. Then the recombinant plasmids shRNA1#, shRNA2#, shRNA3# and shRNA-Neg were transfected into H9c2 cells with liposomes Lipofectamine^{TM} 2000, the cells were divided into control group (untransfected group), shRNA-Neg group (negative control group), and three transfected groups (shRNA1# group, shRNA2# group and shRNA3# group), each group contained three replicates. After transfection, *TauT* mRNA expression level was examined by using real-time quantitative PCR, and the state of cell proliferation was detected by methyl thiazolyl tetrazolium (MTT) assay. The results showed that compared with the negative control group and untransfected group, the shRNA1# plasmid significantly decreased the *TauT* mRNA expression level at 24 h after transfection ($P<0.01$), the shRNA2# plasmid significantly decreased the *TauT* mRNA expression level at 24, 48 and 72 h after transfection ($P<0.01$), and the shRNA3# plasmid significantly decreased the *TauT* mRNA expression level at 72 h after transfection ($P<0.01$). The MTT detected result showed that the cell proliferation in the transfected cells was not significantly affected by shRNAs plasmid compared with untransfected cells and negative control cells at 24, 48, 72 h ($P>0.05$). The present study indicates that the shRNA2# recombinant plasmid can effectively inhibit the expression of *TauT* gene and does not affect the proliferation of H9c2 cells at 72 h after transfection, which will be of benefit to the further study on the functions of TauT and taurine in cardiocytes metabolism.

Keywords： H9c2 cell, RNAi, TauT, cell proliferation

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