[1]韩晓凤,李美玲,张鹏,等.siIRE1α重组腺病毒对内质网应激介导凋亡的影响[J].第三军医大学学报,2014,36(11):1147-1152.
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siIRE1α重组腺病毒对内质网应激介导凋亡的

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Title: Recombinant adenovirus siIRE1α inhibits endoplasmic reticulum stress-mediated apoptosis *in vitro*

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摘要: 目的 构建内质网跨膜蛋白肌醇酶1α（inositol-requiring enzyme 1α，IRE1α）基因干扰RNA（small interfering RNA，siRNA）重组腺病毒，并探讨内质网应激（endoplasmic reticulum stress，ERS）状态下其对C2C12细胞增殖凋亡的影响。 方法 人工合成靶向IRE1α的siRNA序列，连接到穿梭载体pSES-HUS上，与腺病毒骨架质粒pAdeasy-1在大肠杆菌BJ5183感受态中进行同源重组，得到pAdSES-HUS-IRE1α siRNA重组质粒。通过脂质体介导在HEK293细胞中包装并扩增重组腺病毒Ad-IRE1α siRNA，在C2C12细胞中采用RT-PCR和Western blot检测其干扰效果，并通过FCM法和MTT检测Tm诱导ERS时病毒对C2C12细胞增殖凋亡的影响（分为4组：NC组、Tm单独处理组、Tm+Ad-RFP组和Tm+Ad-IRE1α siRNA组），Western blot检测C2C12细胞中Cleaved Caspase-3和Chop蛋白的表达。 结果 成功获得了病毒滴度约为4.3×10^{11} PFU/mL的重组腺病毒Ad-IRE1α siRNA。RT-PCR和Western blot检测结果表明，该重组腺病毒有效地抑制了C2C12细胞中IRE1α的表达。FCM检测结果表明，ERS条件下，Tm+Ad-IRE1α siRNA组S期的细胞比例分别比Tm单独处理组和Tm+Ad-RFP组升高12.62%和14.80%

（*P*<0.05）；凋亡率比Tm单独处理组和Tm+Ad-RFP组降低16.64%和16.26%（*P*<0.05）。MTT实验结果与Cleaved Caspase-3和Chop蛋白的表达与FCM结果一致。　　结论　　重组腺病毒Ad-IRE1α siRNA在C2C12细胞中能有效抑制IRE1α的表达；ERS状态下，RNA干扰IRE1α基因可促进C2C12细胞的增殖，抑制其凋亡。

Abstract: Objective To construct the adenovirus vector containing small interfering RNA (siRNA) targeted against human inositol-requiring enzyme 1α (IRE1α) gene, and determine its effect on the proliferation and apoptosis of mouse C2C12 myoblasts C2C12 under endoplasmic reticulum (ER) stress. Methods The siRNA sequence targeting IRE1α gene was synthesized and cloned into the shuttle plasmid pSES-HUS to generate the vector pSES-HUS-IRE1α siRNA, which was later homogenously recombined with the adenovirus backbone plasmid pAdEasy-I in *E. coli* BJ5183. Then the recombinant adenovirus was transfected into the 293 packing cells by lipofectamine-mediated transfection to amplify the recombinant adenovirus Ad-IRE1α siRNA. The C2C12 cells were infected with this adenovirus, and the expression of IRE1α at mRNA and protein levels were detected by RT-PCR and Western blotting respectively. The effects of the recombinant adenovirus on the proliferation and apoptosis of C2C12 after the treatment of ER stress inducer Tm were detected by flow cytometry and MTT assay. The normal control, and the cells treated by the Tm, Tm+Ad-RFP served as control. The expression levels of cleaved Caspase-3 and chop were detected by Western blotting. Results The recombinant adenovirus Ad-IRE1α siRNA with high titer of 4.3×10^{11} PFU/mL was successfully obtained. Both the IRE1α mRNA and protein levels were significantly decreased in the C2C12 cells after 48 h of infection with Ad-IRE1α siRNA. Flow cytometry showed that in the stress condition the C2C12 cells infected by Ad-IRE1α siRNA had more cells at S phase than the cells treated by Tm and Tm+Ad-RFP (increased by 12.62% and 14.80% respectively, *P*<0.05), and the apoptotic rate of the Ad-IRE1α siRNA group was decreased by 16.64% and 16.26% (*P*<0.05) when compared with the 2 control groups. MTT assay and Western blotting results of cleaved Caspase-3 and chop were in accordance with those results shown by FCM. Conclusion The recombinant adenovirus Ad-IRE1α siRNA significantly decreases the expression of IRE1α at mRNA and protein levels in C2C12 cells. Infecting C2C12 cells with Ad-IRE1α siRNA promotes the proliferation and suppresses the apoptosis in the cells under the stress condition.

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