

[1]黄士隋,史良会,黄广岩.RNA干扰下调LAT1表达对胃腺癌SGC-7901细胞增殖、侵袭、转移及细胞周期的影响[J].第三军医大学学报,2013,35(04):320-324.

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RNA干扰下调LAT1表达对胃腺癌SGC-7901细胞增殖、侵袭、转移及

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Title: Effect of RNA interference targeting LAT1 on proliferation, migration and invasion of SGC7901 cells

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摘要: 目的 观察下调胃癌细胞SGC-7901的LAT1表达对其体外增殖、迁移、侵袭的影响。方法 设计并合成2条针对编码LAT1的SLC7A5 mRNA寡核苷酸干扰序列和阴性对照序列,连接pGPU6/GFP/Neo质粒载体上,分别转染至细胞株SGC-7901, RT-PCR与Western blot检测干扰后LAT1、LAT1异二聚体CD98hc mRNA与蛋白表达量,筛选出抑制率较高的质粒,用G418筛选出稳定表达干扰序列的胃癌细胞株。MTT法检测细胞增殖,流式细胞仪检测细胞周期,Transwell小室检测细胞迁移与侵袭能力。结果 酶切电泳分析和DNA测序证实成功构建LAT1-shRNA质粒,转染48 h后,与空白对照组和阴性对照质粒转染组相比, LAT1-shRNA1与LAT1-shRNA2重组载体转染胃癌 SGC-7901细胞后对LAT1 mRNA表达及LAT1蛋白表达均有抑制作用, LAT1-shRNA2重组载体抑制效率最高,但CD98hc未见明显变化。与空白对照组和阴性对照转染组比较, LAT1的表达下调后,胃癌细胞SGC-7901的增殖、迁移与侵袭能力均受到一定程度的抑制 ($P<0.05$)。流式细胞术检测结果表明,细胞阻滞于G₀/G₁期。结论 下调LAT1的表达对胃癌细胞SGC-7901的体外增殖、迁移与侵袭有抑制作用,并且细胞周期被阻滞, LAT1在人胃癌细胞增殖、迁移与侵袭中发挥重要作用。

Abstract: Objective To investigate the effect of downregulation of LAT1 on the proliferation, migration, and invasion of SGC7901 cells. Methods Two sets of shRNA targeting LAT1 and one scramble shRNA were synthesized and inserted into pGPU6/GFP/Neo plasmid vectors, respectively. Then the recombinant plasmids containing the shRNAs were transferred into SGC7901 cells and the knockdown efficiency was determined by using RT-PCR and Western blotting to detect the mRNA and protein expression levels of LAT1 and CD98hc, which forms the heterodimer with LAT1. The cells with higher knockdown efficiency were subjected to a subsequent selection using G418 to establish stable cell lines with constitutive lower expression of LAT1 compared with the cells transfected with the recombinant plasmids containing scramble shRNA. MTT assay, flow cytometry, and Transwell assay were conducted to determine the potency of proliferation, cell cycle, migration and invasion of SGC7901 cells. Results The sequences of LAT1-shRNA recombinant plasmids were identified by restriction endonuclease analysis and DNA sequencing. The mRNA and protein levels of LAT1 in SGC7901 cells decreased after LAT1-shRNA1 and LAT1-shRNA2 transfection for 48 h compared with the cells with scramble shRNA transfection. LAT-shRNA2 had higher knockdown efficiency than LAT-shRNA1 did. However, the expression of CD98hc was not affected. Downregulation of LAT1 inhibited the proliferation, migration and invasion of SGC7901 cells ($P<0.05$). Flow cytometry showed SGC7901 cells were arrested in G₀/G₁ phase. Conclusion Downregulation of LAT1 inhibits the proliferation, migration and invasion of SGC7901 cells. LAT1 plays an important role in the proliferation, migration and invasion of gastric cancer cells.

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