[1]邵新宏,于游,张才全.miR-34a靶向调控NOTCH1基因对SW480细胞增殖的影响[J].第三军医大学学报,2012,34(22):2297-2301.
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miR-34a靶向调控NOTCH1基因对SW480细胞增殖的

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Title: Inhibitory effects of miR-34a on NOTCH1 gene expression and SW480 cell proliferation

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关键词: 微小RNA; NOTCH1基因; 靶向调节; 细胞增殖; 结直肠癌

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摘要: 目的 探讨miR-34a靶向调控NOTCH1基因表达而对结肠癌SW480细胞增殖的影响。 方法 通过生物信息学预测，NOTCH1为miR-34a特异性靶基因。构建含miR-34a 结合位点的NOTCH1基因3′-UTR域荧光素酶报告载体。通过荧光素酶报告载体系统检测miR-34a与NOTCH1的3′-UTR相互作用对荧光素酶活性的影响；免疫印迹技术检测miR-34a对NOTCH1蛋白表达的影响。采用MTT法及流式细胞检测转染miR-34a对SW480细胞增殖的影响。 结果 经过酶切及基因测序鉴定，NOTCH1基因3′-UTR 序列的双荧光素酶报告重组质粒构建成功；荧光素酶结果显示在SW480细胞中加入miR-34a的类似物和重组载体，荧光素酶的活性是只加入空载体的SW480组53.4%（*P*=0.003 8）;而在HEK293细胞中加入miR-34a的抑制物和重组载体，荧光素酶的活性是只加入空载体的HEK293组145%（*P*=0.002 1）,说明miR-34a有与NOTCH1的3′-UTR位点相结合。免疫印迹结果显示在SW480细胞中加入miR-34a的类似物，NOTCH1蛋白的表达水平是未处理SW480组下降53.6%（*P*<0.05）;而在HEK293细胞中加入miR-34a的抑制物，NOTCH1蛋白的表达水平较未处理HEK293组升高78.9%（*P*=0.03），说明miR-34a负性调控NOTCH1蛋白的表达。miR-34a过表达的SW480细胞较未处理的SW480的生长速度明显减慢（*P*<0.05），且阻滞在G_0~G_1期，说明miR-34a过表达后能抑制SW480细胞增殖。 结论 miR-34a负性靶向调控NOTCH1基因的表达而抑制SW480细胞的增殖。

Abstract: Objective To analyze the effect of miR-34a on NOTCH1 gene expression and SW480 cell proliferation. Methods NOTCH1 was predicted to be the specific target gene of miR-34a by bioinformatics. The dual luciferase vector

containing 3′ -UTR of NOTCH1 gene was constructed, and the 3′ -UTR was regarded as the binding site of miR-34a. The effect of miR-34a interaction with the 3′ -UTR of NOTCH1 on luciferase activity was detected with a dual luciferase assay system. The expression level of NOTCH1 protein affected by miR-34a was detected by Western blotting. The proliferation of SW480 cells transfected with miR-34a was measured by MTT assay and flow cytometry. Results The dual luciferase recombinant vector containing the 3′ -UTR of NOTCH1 gene was successfully constructed and verified by enzyme digestion and gene sequencing. The luciferase activity significantly reduced to 53.4% in the SW480 cells co-transfected with the recombinant vectors and miR-34a mimics as compared with the SW480 cells transfected with empty vectors (*P*=0.003 8), while the luciferase activity significantly was enhanced to 145% in the HEK293 cells co-transfected with the recombinant vectors and miR-34a inhibitors as compared with the HEK293 cells transfected with empty vectors (*P*=0.002 1). The results of the luciferase assay revealed that miR-34a could negatively regulate the luciferase activity by interacting with the 3′ -UTR of NOTCH1. Western blotting results demonstrated that the NOTCH1 protein level in the SW480 cells transfected with miR-34a mimics decreased by 53.6% as compared with the untreated SW480 cells (*P*<0.05), while the NOTCH1 protein level in the HEK 293 cells transfected with miR-34a inhibitors increased by 78.9% as compared with the untreated HEK293 cells (*P*=0.03). NOTCH1 protein expression was negatively regulated by miR-34a. The growth of SW480 cells transfected with miR-34a was much slower than that of the untreated SW480 cells, and the cells were arrested at G_0-G_1 phase, suggesting miR-34a overexpression could inhibit the proliferation of SW480 cells. Conclusion miR-34a negatively targetedly regulates the expression of NOTCH1 and inhibits the proliferation of SW480 cells.

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