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抑制miR-21表达对结肠癌HCT116细胞生物学行为的影响

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摘要：

目的：探讨抑制 miR-21 表达对结肠癌HCT116细胞增殖、周期、凋亡、侵袭和迁移等生物学行为的影响。方法：实验分为3组，以miR-21抑制剂转染HCT116细胞为转染抑制组（IN），另设阴性对照组（NC）、空白对照组（MOCK），以Real-time PCR检测转染后HCT116细胞中miR-21的表达，应用MTT法、流式细胞术、Transwell侵袭和迁移实验检测转染后HCT116细胞的增殖、周期、凋亡、侵袭、迁移；以Western blotting检测转染后HCT116细胞PTEN的表达，荧光素酶报告实验检测转染后HCT116细胞PTEN的活性。结果：miR-21抑制剂转染后，HCT116细胞中miR-21的表达较NC和MOCK组细胞明显降低。下调miR-21后，HCT116细胞的增殖能力明显降低\[72 h时：（1.05±0.45） vs （1.43±0.02），（1.45±0.01）；t =13.83, P =0 000 159；t =14.88, P =0.000 119\]，细胞凋亡率显著增加\[（16.30±1.00）% vs （1.87±0.53）%，（1.86±0.12）%；t =25.01, P =0.000 015 2；t =24.985, P =0.000 015 2\]，细胞周期阻滞于G 0/G 1期，细胞的侵袭\[（50±2.0） vs （115±3.0），（111±3.0）个；t =29.09, P =0.000 0 008 31；t =31.23, P =0.000 006 27\]和迁移能力\[（22±2.0） vs （52.3±2.5），（53.0±1.0）个；t =24 01, P =0.000 017 8；t =16.34, P =0.000 082 0\]明显下降。miR-21抑制剂转染的HCT116细胞中PTEN的表达及其荧光素酶相对活性均显著增加。结论：miR-21可能通过抑制PTEN进而调控大肠癌细胞生物学行为，PTEN可能是miR-21的靶基因之一。

关键词：miR-21 大肠癌 生物学行为 PTEN

Impact of inhibition of miRNA-21 on biological functions of colorectal cancer cells

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Abstract:

Objective : To determine the effects of microRNA-21 (miR-21) inhibition on the various functional aspects (e.g., proliferation, apoptosis, cell cycle and invasion and migration) of colon cancer HCT116 cells in vitro . Methods: HCT116 cells were transfected with an miR-21 inhibitor, a non-sequence specific inhibitor as a negative control and the transfection reagent as a mock control, respectively. The level of miR-21 in transfected HCT116 was determined by Real-time PCR. The Proliferation, apoptosis, cell cycle progression, invasion and migration of the transfectants were evaluated by methyl thiazolyl tetrazolium (MTT) assay, flow cytometry (FCM), transwell invasion and migration assays, respectively. Protein level of phosphatase and tensin homolog (PTEN) and PTEN promoter activity in HCT116 cells after transfection were evaluated by Western blotting analysis and the luciferase reporter assay, respectively. Results: The level of miR-21 was significantly lower in HCT116 cells transfected with the miR-21 inhibitor than in cells transfected with non-specific inhibitor or mock. At 72 hours after transfection, the miR-21 inhibitor significantly suppressed HCT116 cell proliferation (1.05±0.45 vs 1.43±0.02 and 1.45±0.01, P <0.001), significantly increased HCT116 cell apoptosis (\[16.30±1.00\]% vs \[1.87±0.53\]% and \[1.86±0.12\]%, P <0.0001) and HCT cell cycle arrest in G 0/G 1 phase, and significantly decreased the invasion (50±2.0 vs 115±3.0 and 111±3.0, P <0.0001) and migration abilities (22±2 0 vs 52.3±2.5 and 53.0±1.0, P <0.0001), as compared with the two controls. Moreover, miR-21 inhibition resulted in remarkable increases in protein levels and activity of PTEN in HCT116 cells. Conclusion: MicroRNA21 may regulate proliferation/apoptosis, invasion and migration of colorectal cancer cells, possibly through modulating the expression and activity of PTEN.

Keywords:miR-21 colorectal cancer biological behavior PTEN

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