[1]张丹,郝宁波,唐波,等.miR-1182调控胃癌细胞人端粒酶反转录酶的分子机制及其对迁移能力的影响[J].第三军医大学学报,2014,36(10):1074-1077.

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miR-1182调控胃癌细胞人端粒酶反转录酶的分子机制及其对迁移能力的影响(PDF)

分享到：

《第三军医大学学报》[ISSN:1000-5404/CN:51-1095/R] 卷: 36 期数: 2014年第10期 页码: 1074-1077 栏目: 论著 出版日期: 2014-05-30

Title: Mechanism of miR-1182 regulating human telomerase reverse transcriptase in gastric cancer cells and its effect on cell migration capability

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第三军医大学新桥医院消化内科

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关键词: 端粒酶反转录酶; miR-1182; 胃肿瘤; 肿瘤转移

Keywords: human telomerase reverse transcriptase ; miR-1182; gastric cancer; tumor metastasis

分类号: R345; R730.23; R735.2

文献标志码: A

摘要: 目的 研究miR-1182在胃癌细胞中调控人端粒酶反转录酶（human telomerase reverse transcriptase，hTERT）的机制及对其迁移能力的影响。 方法 胃癌细胞MKN28中转染miR-1182 类似物，qRT-PCR检测转染后细胞miR-1182的相对表达量，Western blot检测转染后细胞hTERT的表达变化；进一步通过生物信息学预测miR-1182与hTERT mRNA的结合位点，双荧光素酶实验分析miR-1182对hTERT mRNA的作用机制；Transwell实验检测转染miR-1182类似物对MKN28细胞体外迁移能力的影响。 结果 qRT-PCR表明miR-1182组的miR-1182的相对表达量（10.168±2.645）明显高于对照组（1.008±0.167）（*P*<0.01）。Western blot结果显示在胃癌细胞系中过表达miR-1182后，hTERT的蛋白水平下调。双荧光素酶实验表明miR-1182可与hTERT mRNA的ORF区结合，且其主要结合部位为hTERT mRNA的ORF-1；在Transwell迁移实验中，miR-1182类似物转染细胞后，miR-1182组穿膜细胞数为（23.333±4.509）/视野，对照组穿膜细胞数为（71.000±4.582）/视野，miR-1182组细胞迁移能力明显低于对照组（*P*<0.01）。 结论 miR-1182通过与胃癌细胞hTERT的 ORF区结合，从而在转录

后水平抑制hTERT的表达，并发现其可抑制胃癌细胞的迁移能力。

Abstract: Objective To investigate the mechanism of miR-1182 regulating human telomerase reverse transcriptase (hTERT) in gastric cancer cells and to study the effect of miR-1182 on the migration capability of gastric cancer cells. Methods MKN28 gastric cancer cells were transfected with miR-1182 mimics. Quantitative RT-PCR was used to test the level of miR-1182, and Western blot analysis to detect the expression of hTERT. Bioinformatic analysis was carried out to predict the possible binding sites of miR-1182 to hTERT mRNA. Dual-luciferase assay was adopted to analyze the mechanism of miR-1182 acting on hTERT. Transwell migration assay was used to analyze cell migration ability. Results The results of quantitative RT-PCR indicated that the relative expression of miR-1182 in the miR-1182 mimics treatment group was higher than that in the control group (10.168±2.645 *vs* 1.008±0.167, $P<0.01$). Western blot results suggested that over-expression of miR-1182 could inhibit the expression of hTERT. Dual-luciferase assay results suggested that miR-1182 bond to the open reading frame(ORF) of hTERT, with ORF-1 as the main target site. In Transwell migration assay, the cells passing through the membrane were 23.333±4.509 in visual field in the miR-1182 mimics treatment group and 71.000±4.582 in the control group. The migration ability of the cells in the miR-1182 mimics treatment group was significantly lower than that in the control group ($P<0.01$). Conclusion MiR-1182 down-regulates hTERT expression by binding to its ORF, and also inhibits the migration capability of gastric cancer cells.

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更新日期/Last Update: 2014-05-23