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miRNA-486-5p对胃癌细胞SGC7901中NRP2表达的影响

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摘要：

预测并鉴定miRNA-486-5p在人胃癌细胞SGC7901中的靶基因及其表达。方法：采用生物信息学技术预测miRNA-486-5p的作用靶点，构建miRNA-486-5p过表达质粒（GV214-miR）并转染入SGC7901细胞（SGC7901-miR）中，以空质粒转染SGC7901细胞（SGC7901-miR-NC）为阴性对照，以SGC7901细胞为空白对照。Real-time PCR检测转染细胞中miRNA-486-5p及其靶基因神经纤毛蛋白2（neuropilin-2，NRP2）mRNA的表达，Western blotting检测NRP2的表达，双荧光素酶实验验证miRNA-486-5p对NRP2基因的调控机制。结果：经生物信息学预测，选择与胃癌生物学行为密切相关的NRP2作为miRNA-486-5p的靶基因。与空白组相比，GV214-miR转染后的SGC7901细胞miRNA-486-5p表达显著上调[（8.21±1.18） vs（1.02+0.26），P<0.01]，NRP2 mRNA表达无明显变化（P>0.05），而NRP2蛋白表达则明显下调[（0.36±0.06） vs （0 76±0 05），P<0.05]，双荧光素酶实验证实miRNA-486-5p可与NRP2 mRNA 3′-UTR直接结合，从而发挥对NRP2转录后翻译的抑制作用。结论：miRNA-486-5p在胃癌细胞SGC7901中可直接作用于NRP2 mRNA 3′ UTR，从而抑制其表达。

关键词：miRNA-486-5p 胃癌 生物信息学技术 神经纤毛蛋白2 转染

Effect of miRNA-486-5p on expression of NRP2 in gastric carcinoma SGC7901 cells

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Abstract:

To predict and identify the target genes of microRNA-486-5p (miRNA-486-5p) in human gastric cancer SGC7901 cells. Methods: Possible target genes of miRNA-486-5p were predicted by bioinformatics techniques and accordingly miRNA-486-5p over-expressing plasmid (GV214-miR) against the identified target gene, neuropilin-2 (NRP-2) was constructed. SGC7901 cells were transfected with a control miRNA and an NRP-2-specific miRNA-486-5p. In the transfectants and non-transfected control cells, miRNA-486-5p and NRP-2 mRNA levels and NRP-2 protein levels were analyzed by real-time PCR and Western blotting respectively, and the NRP-2 promoter activity was evaluated by a dual luciferase reporter assay. Results: The expression of miRNA-486-5p in miRNA-486-5p-transfected SGC7901 cells (SGC7901-miR cells) was significantly up-regulated compared with that in the control group (8.21±1 18 vs 1.02±0 26, P<0.01). No significant difference in NRP2 mRNA abundance was observed (P>0.05). However, the NRP2 protein level was significantly reduced in SGC7901-miR cells (0.36±0.06) as compared with SGC7901 cells transfected with the control plasmid (0.76±0.05, P<0.05). Dual luciferase reporter assay demonstrated that miRNA-486-5p directly targeted the 3’-untranslated region (UTR) of the NRP2 gene, resulting in inhibition of the post-transcriptional translation of NRP2. Conclusion: Sequence-specific miRNA-486-5p may suppress the expression of NRP2 at the protein level in human gastric cancer cells by binding to NRP2 mRNA 3’ UTR directly.

Keywords:miR- 486-5p gastric carcinoma bioinformatics technique neuropilin-2(NRP2) transfect

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