

[1]金俊余,孙建国,张岸梅,等.mmu-miR-294调控MMP3靶基因对LLC细胞侵袭迁移的影响[J].第三军医大学学报,2013,35(12):1200-1204.

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Title: Effect of mmu-miR-294 regulating its target gene MMP3 on invasion and migration in mouse lung cancer stem cells

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关键词: [mmu-miR-294](#); [肺癌干细胞](#); [肺癌](#); [MMP3](#)

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摘要: 目的 预测并验证小鼠mmu-miR-294 调控的靶基因, 探讨其在肺癌发生发展中的生物学功能。 方法 生物信息学预测mmu-miR-294可能调控的靶基因金属蛋白酶(MMP3), 双荧光素酶检测验证mmu-miR-294调控MMP3的真实性; 脂质体2000介导转染mmu-miR-294模拟物进入Lewis (LLC) 细胞株, 通过Transwell实验检测细胞侵袭、迁移能力的改变。 结果 重组质粒经Xba I 单酶切能获得约5 000 bp和100 bp的酶切片段, 阳性克隆测序, 双荧光素酶报告基因检测证明合成寡核苷酸链序列插入正确; 脂质体2000介导转染mmu-miR-294模拟物, 过表达实验组MMP3蛋白水平较对照组明显降低。转染mmu-miR-294模拟物后LLC细胞的侵袭迁移能力显著降低($P<0.01$)。 结论 低表达mmu-miR-294有助于维持LLC的侵袭转移特性, 增加其表达水平可以有效抑制LLC的侵袭迁移能力。mmu-miR-294可能通过调控其靶基因MMP3表达而发挥功能。

Abstract: Objective To predict and validate mmu-miR-294 target gene in mouse lung cancer stem cells, and to investigate its biological functions in the

carcinogenesis and development of lung cancer. Methods Bioinformatics analysis predicted that mmu-miR-294 might regulate targeted genes like matrix metalloproteinase 3 (MMP3). The sequence of MMP3 was artificially synthesized, and the seed sequence containing 3' -UTR was directly inserted to an eukaryotic expression plasmid pGL3-promoter by *Xba* I digestion. *E. coli* DH5 alpha was transformed. Positive clones were identified by enzyme digestion and DNA sequencing, and dual luciferase report assay was applied for validation. mmu-miR-294 mimics was transferred into Lewis cells mediated by Liposome 2000. The expression level of miR-294 was measured by real-time quantitative PCR, and Western blotting was used to detect the expression level of MMP3 protein.

Results The 5 000 bp and 100 bp restriction fragments were obtained after the recombinant plasmids were digested by *Xba* I . The positive clones were identified by sequencing and dual luciferase report assay. Compared with the control group, the expression level of MMP3 protein was significantly decreased in the mmu-miR-294 mimics transfection group, and the invasion and migration abilities of the Lewis cells was reduced significantly ($P<0.01$).

Conclusion Up-regulation of mmu-miR-294 can effectively inhibit the invasion and migration of Lewis cells through down-regulating the expression of its target gene MMP3.

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金俊余, 孙建国, 张岸梅, 等. mmu-miR-294调控MMP3靶基因对LLC细胞侵袭迁移的影响[J]. 第三军医大学学报, 2013, 35(12):1200-1204.

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