miR-194通过靶定DNMT3A基因调控肝癌细胞的生长

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Title: **Regulation of miR-194 on growth of hepatocellular carcinoma cell through targeting DNMT3A**

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关键词: 微小RNA-194; HepG2细胞; 增殖; 细胞周期; 甲基化转移酶3A

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摘要: 目的:探讨miR-194对肝癌细胞增殖和细胞周期的影响及其潜在的作用机制。方法：实时定量聚合酶链式反应检测肝癌细胞系HepG2和正常肝细胞系L-O2中miR-194的表达水平。构建miR-194过表达质粒，采用MTT法检测细胞增殖活力,流式细胞术检测细胞周期；双荧光素酶报告基因分析法预测和验证miR-194可能的靶基因。结果：实时定量PCR结果显示，miR-194在肝癌细胞中的表达明显低于肝脏正常细胞。在肝癌细胞中过表达miR-194抑制细胞生长。而流式细胞术检测发现细胞周期进程减慢，G1期比例增加，S期比例相应的减少。靶基因筛选得到DNMT3A为miR-194的候选靶基因。荧光报告载体实验证实，miR-194能够通过作用于靶基因3' 非翻译区的特定位点，对其表达在转录后进行负性调节。而在miR-194表达增加的肝癌细胞中，靶基因的mRNA表达水平和蛋白表达水平都有明显降低。在肝癌细胞HepG2中，敲除靶基因DNMT3A后，细胞的增殖能力减弱，相反，当把DNMT3A过表达后，细胞的增殖能力增强，可以挽救miR-194对细胞的表型影响。结论：miR-194可通过靶定DNMT3A基因抑制肝癌细胞的生长。

Abstract: Objective:To explore effect of miR-194 on proliferation and cell cycle of the human hepatocellular carcinoma cell and its potential mechanism.Methods:Expressions of miR-194 in hepatoma cell line HepG2 as well as normal liver cell L-O2 were detected by RT-PCR.The plasmid with over-expression of miR-194 was constructed.MTT and FACS were respectively used to measure proliferation ability of the cells and cell cycle.Possible target gene of miR-194 was forecasted and verified with dual luciferase report gene assay.Results:Compared with normal liver cell,miR-194 is low-expressed in HepG2.Over-expression of miR-194 decreased cell growth.FACS showed miR-194 arrested cells cycle in G1 phase.The DNMT3A was identified to be a putative target gene,whose mRNA 3'-untranslated region (3' UTR) contains the potential binding site of miR-194.The fluorescent reporter experiment also confirmed that miR-194 can directly bind to the target genes mRNA 3' UTR.The mRNA and protein level of DNMT3A in HepG2 gave the clue that miR-194 can negatively regulate the genes expression through mRNA decay.Knockdown of DNMT3A by RNA interference can decrease the cell proliferation.Finally,when cells were transfected with DNMT3A and miR-194,the cell proliferation ability was significantly recovered comparing to the cells transfected with pcDNA and miR-194.Conclusion:miR-194 could inhibit proliferation and cell cycle of the hepatocellular carcinoma cell through targeting DNMT3A.

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