

论著

DNA 甲基化对NOR1 基因启动子活性和基因表达的影响

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摘要:

目的:研究DNA 甲基化对NOR1 基因启动子活性和表达的影响。方法:采用SssI 甲基转移酶处理NOR1基因启动子报告载体,经重亚硫酸钠修饰后测序检测报告载体甲基化水平。未经SssI 处理和经SssI 处理的NOR1 基因启动子报告载体分别转染细胞,检测荧光素酶活性或GFP 表达。采用去甲基化药物5-氮杂-2'-脱氧胞苷处理白血病细胞系HL60 细胞,抽提RNA,Real-time RT-PCR 检测NOR1 mRNA 表达水平。结果:重亚硫酸钠测序表明SssI 甲基转移酶体外处理导致NOR1 启动子CpG 岛CG 位点完全甲基化。体外甲基化的NOR1 启动子活性完全丧失。去甲基化药物5-氮杂-2'-脱氧胞苷连续处理3 d 后,白血病细胞系HL60 细胞中内源性NOR1 mRNA 表达水平显著增加。结论:SssI 甲基转移酶可用于体外甲基化修饰NOR1 基因启动子报告载体。甲基化修饰导致NOR1 启动子活性丧失。去甲基化药物5-氮杂-2'-脱氧胞苷处理恢复NOR1 mRNA 表达。

关键词: 表观遗传学 甲基化 5-氮杂-2'-脱氧胞苷 NOR1 启动子 表达

Effect of DNA hypermethylation on NOR1 promoter activity and expression

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Abstract:

Objective: To analyze the effect of DNA hypermethylation on NOR1 promoter activity and expression. Methods: NOR1 promoter plasmids were treated with SssI methyltransferase. The plasmids were modified by sodium bisulfite and purified. Sodium bisulfite-modified plasmids were subjected to PCR with primers designed to analyze the methylation status of 26 CpG sites in a 311-bp region of the NOR1 promoter. Cells were transfected by methylated or mock-methylated promoter plasmids. The promoter activities were assessed by the luciferase levels of cell lysates or by directly observing GFP expression under fluorescence microscope. HL60 cells were treated with different concentrations of 5-aza-dC. Total RNA was isolated from harvested cells. Real-time RT-PCR was used to measure the expression level of NOR1 mRNA. Results: Bisulfite sequencing confirmed that SssI methyltransferase treatment successfully resulted in intensive hypermethylation of the NOR1 promoter plasmids. The promoter activity of NOR1 promoter plasmids was totally blocked by SssI methyltransferase treatment. NOR1 expression levels in HL60 cells were restored by 5-aza-dC treatment. Conclusion: NOR1 promoter plasmids are intensively hypermethylated by SssI methyltransferase treatment. The promoter activity of NOR1 promoter plasmids are totally blocked by SssI methyltransferase treatment. The 5-aza-dC treatment may restore the endogenous NOR1 mRNA level in HL60 cells.

Keywords: epigenetics methylation 5-aza-dC NOR1 promoter expression

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