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论著

启动子区5′CpG岛去甲基化对人结肠癌细胞生物学表型的影响

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摘要 目的：探讨DNA启动子区5′CpG岛甲基化状态与人结肠癌RKO细胞增殖凋亡等生物学特征的关系。方法： 应用特异性DNA甲基转移酶（DNMTs）抑制剂-5-氮-2′-脱氧胞苷（5-Aza-2′-deoxycytidine，5-Aza-CdR）处理肠癌RKO细胞72 h，甲基化特异性PCR（methylation-specific PCR,MSP）及DNA测序法分析p16/CDKN2基因CpG岛甲基化状态；MTT、FCM、荧光染色及透射电镜检测启动子区去甲基化后细胞生长、形态和细胞周期凋亡的影响。 结果： DNMTs抑制剂能较好地逆转启动子区胞嘧啶甲基化状态；CpG岛去甲基化后能明显地抑制肠癌细胞的生长，增加细胞群体倍增时间（P<0.01），诱导肠癌细胞凋亡，影响肠癌细胞周期分布，并具有良好的量效依赖关系。 结论： 通过逆转CpG岛高甲基化能有效地抑制肠癌细胞增殖，为临床治疗大肠癌提供新的作用靶点。

关键词 甲基化； 结直肠肿瘤； 细胞凋亡； 细胞周期； 脱氧胞苷

分类号 R730.2 R730.5

Effects of promoter region 5′CpG island demethylation on biological phenotype in human colorectal cancer cells

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Abstract

<FONT face=Verdana>AIM: To explore the relationship between methylation status of promoter region 5′CpG island and the biological phenotype in human colorectal cancer RKO cell lines. METHODS: RKO cells were treated with selective DNA methyltransferase (DNMTs) inhibitor, 5-Aza-2′-deoxycytidine (5-Aza-CdR), for 72 h. Methylation-specific PCR (MSP), T-A clone and DNA sequence analysis were used to detect 5′CpG island methylation status of p16/CDKN2 tumor suppresor gene. Cell growth, cell cycle arrest and apoptosis were analyzed by MTT, flow cytometry (FCM), fluorescent dye staining and transmission electron microscope. RESULTS: DNMTs inhibitor (5-Aza-CdR) effectively reversed the hypermethylation status of 5′ CpG island. The effects of 5-Aza-CdR on cell growth inhibition (P<0.01), apoptosis and cell cycle arrest were observed in a dose-dependent manner. CONCLUSION: Selective DNMTs inhibitor inhibits cell growth by 5′CpG island demethylation, and this may be a potential new therapeutic target for colorectal cancer.</FONT>

Key words Methylation Colorectal neoplasms Apoptosis Cell cycle Deoxycytidine

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