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论著

# 甲基化抑制剂5-杂氮胞苷对T淋巴细胞株程序性死亡受体-1基因启动子区域甲基化水平及其表达的影响

张旻[1], 肖新强[1], 梁云生[2], 彭敏源[3], 蒋永芳[1], 许允[1], 龚国忠[1]

1. 湘雅二医院感染科, 长沙 410011;
2. 湘雅二医院表观遗传学 研究中心, 长沙 410011;
3. 湘雅医院血液科, 长沙 410008

**摘要:**

**目的**:以T淋巴细胞株Molt-4细胞为模型,探讨甲基化抑制剂5-杂氮胞苷(5-azacytidine,5-Zac)对淋巴细胞表面程序性死亡受体-1(programmed death receptor 1, *PD-1* )基因启动子的去甲基化作用及其诱导的 *PD-1* 基因表达的改变,并进一步研究去甲基化作用与 *PD-1* 基因表达之间的关系。**方法**:以不同浓度的5-Zac分组(0 μmol/L组、5 μmol/L组、10 μmol/L组)作用于体外培养的Molt-4细胞72 h,流式细胞仪(flow cytometry,FCM)检测细胞表面表达PD-1的Molt-4细胞比例和细胞凋亡率;反转录-聚合酶链反应(reverse transcription polymerase chain reaction, RT-PCR)检测5-Zac作用后 *PD-1* 基因mRNA的转录水平;亚硫酸氢钠处理各组Molt-4细胞DNA,PCR扩增 *PD-1* 启动子基因片段,转化感受态大肠杆菌,挑克隆测序,检测扩增的 *PD-1* 启动子片段甲基化状态。**结果**:0 μmol/L组、5 μmol/L组、10 μmol/L组的5-Zac作用于Molt-4细胞72 h后,PD-1在细胞表面的表达率分别为(1.13±0.01)%,(18.96±1.87)%和(63.09±6.25)%,并呈现浓度依赖性; *PD-1* 基因mRNA表达量显著增加;细胞凋亡检测结果显示:与0 μmol/L组相比,5 μmol/L组、10 μmol/L组5-Zac处理72 h后Molt-4细胞的凋亡率显著增加,0 μmol/L组、5 μmol/L组、10 μmol/L组凋亡率分别为(1.9±0.06)%,(8.98±1.36)%和(24.5±3.68)%,差异有统计学意义($P$<0.01);上述3组DNA亚硫酸氢钠测序结果表明:加入甲基化抑制剂5-Zac处理后, *PD-1* 启动子上-601 bp和-553 bp CpG点去甲基化程度明显增高。**结论**:甲基化抑制剂5-Zac可导致体外培养的T淋巴细胞系Molt-4细胞表面 *PD-1* mRNA 表达显著增加,细胞凋亡率增高,这种增高可能与 *PD-1* 基因启动子区域出现的去甲基化有关。

**关键词:** 程序性死亡受体-1 去甲基化 5-杂氮胞苷 亚硫酸氢钠测序

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## Effect of methylation inhibitor on demethylation pattern of the *PD-1* gene in promoter region and PD-1 expression in human T lymphocyte cell line

ZHANG Min[1], XIAO Xinqiang[1], LIANG Yunsheng[2], PENG Minyuan[3], JIANG Yongfang[1], XU Yun[1], GONG Guozhong[1]

1. Department of Infectious Diseases, Second Xiangya Hospital, Central South University, Changsha 410011;
2. Epigenetics Research Center, Second Xiangya Hospital, Central South University, Changsha 410011;
3. Department of Hematology, Xiangya Hospital, Central South University, Changsha 410008, China

Abstract:

Objective To observe the demethylation effect of demethylation inhibitor 5-azacytidine (5-Zac) on programmed death receptor 1 (PD-1) in Molt-4 cells (T lymphocyte cell line) and to investigate the relationship between DNA demethylation and expression of PD-1. Methods Molt-4 cells were cultured in the medium containing different concentrations of 5-Zac(0, 5, 10 μmol/L) for 72 h. According to the concentrations of 5-Zac, the Molt-4 cells were divided into a 0 μmol/L 5-Zac group, a 5 μmol/L 5-Zac group, and a 10 μmol/L 5-Zac group. The expression of PD-1 in Molt-4 cells was detected by flow cytometry and the apoptosis rate was calculated. The mRNA transcription level of *PD-1* was detected by real-time polymerase chain reaction; Molt-4 cell DNA in all groups were treated by sodium bisulfite. The *PD-1* promoter fragment was amplified by PCR, the amplification fragments were transformed into *E. coli.*, the positive clones were selected for equencing, and the methylation status of the fragments of *PD-1* promoter was examined.Results Seventy-two hours after the 5-Zac treatment, the expression rate of *PD-1* in the Molt-4 cells in the 0 μmol/L 5-Zac group, the 5 μmol/L 5-Zac group, and the 10 μmol/L 5-Zac group was (1.13±0.01)%, (18.96±1.87)%, and (63.09±6.25)% respectively, in a low concentration-dependent way. The *PD-1* mRNA expression level was increased significantly with the 5-Zac treatment. Cells apoptosis showed that:compared with the 0 μmol/L 5-Zac group, the apoptosid rate in the 5 μmol/L 5-Zac group and 10 μmol/L 5-Zac group was signficantly increased, which was (1.9±0.06)%, (8.89±1.36)%, and (24.50±3.68)% in the 0 μmol/L 5-Zac group, the 5 μmol/L 5-Zac group, and the 10 μmol/L 5-Zac mol/L group respectively. The bisulfite genomic sequencing showed that the

demethylation probability of CpG points on -601 bp and -553 bp was significantly increased in the 5-Zac treated cells compared with those untreated. Conclusion 5-Zac can result in the increase of *PD-1* expression in the human lymphoid cell series Molt-4 in vitro, and the apoptosis rate increases, which is related to *PD-1* gene promoter demethylation.

Keywords: programmed death receptor 1 demethylation 5-azacytidine bisulfite genomic sequencing

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**通讯作者**: 龚国忠, E-mail:guozhonggong@yahoo.com

**作者简介**: 张旻,博士研究生,主要从事慢性HBV/HCV感染相关的免疫学发病机制的研究。

**作者**Email: guozhonggong@yahoo.com

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