428~432.氟脲嘧啶促进Egr-1启动子上调人骨髓基质细胞GM-CSF的表达[J].杜 楠,裴雪涛,孙君重,付 艳,赵 晖,王希良.中国肿瘤生物治疗杂志,2008,15(5)

氟脲嘧啶促进Egr-1启动子上调人骨髓基质细胞GM-CSF的表达

点此下载全文

杜 楠 裴雪涛 孙君重 付 艳 赵 晖 王希良

解放军总医院 第一附属医院 肿瘤科，北京 100037;军事医学科学院 野战输血研究所，北京 100037;解放军总医院 第一附属医院 肿瘤科，北京 100037;解放军总医院 第一附属医院 肿瘤科，北京 100037;解放军总医院 第一附属医院 肿瘤科，北京 100037;解放军总医院 第一附属医院 肿瘤科，北京 100037

基金项目：国家自然科学基金资助项目(No.39900040)；解放军总医院第一附属医院新技术重大项目（ZD200502）

DOI：10.3872/j.issn.1007-385X.2008.5.006

摘要：

摘 要 目的: 探索氟脲嘧啶(5-FU)对Egr-1启动子上调人骨髓基质细胞造血因子GM-CSF表达的促进作用，以寻找促进化疗所致造血损伤恢复的方法。方法：构建携带Egr 1调控序列启动的GM-CSF和EGFP双顺反子基因的重组真核表达载体(pCIneo Egr-1 EGFP IRES GM CSF,Egr EG)，通过脂质体转染骨髓基质细胞系HFCL，挑出G418抗性的阳性克隆(HFCL/EG)。采用RT-PCR检测5 FU处理的HFCL/EG细胞GM CSF mRNA表达，用FACS和倒置荧光显微镜观察5 FU诱导HFCL/EG细胞EGFP表达的阳性细胞。在加入5-FU的HFCL/EG细胞培养体系中，用ELISA方法检测GM CSF的含量；将从脐血中分离的单个核细胞接种于5 FU处理后的HFCL/EG培养上清液培养基中，观察其对GM-CFU的增殖作用；采用活性氧抑制剂N-乙酰半胱氨酸检测5-FU通过活性氧诱导Egr-1启动子CArG序列调控下游基因表达的特异性。结果：构建了Egr 1调控序列启动的双顺反子基因表达载体Egr-EG，获得其转染细胞HFCL/EG。在5-FU处理的HFCL/EG细胞中，RT-PCR显示其GM-CSF mRNA表达增强，流式细胞术证实有EGFP的显著表达。在5 FU处理后,HFCL/EG细胞培养上清液GM-CSF含量和GM-CFU形成数量分别较未处理细胞能明显增高（P<0.01）；在5-FU处理的HFCL/EG细胞中，N-乙酰半胱氨酸能明显减少GM CSF含量（P<0.01）。结论：5-FU能促进Egr 1启动子上调人骨髓基质细胞GM-CSF基因的表达，从而对化疗后的造血损伤产生一定的恢复作用。

关键词：early growth response 1(Egr-1) 5-fluorouracil granulocyte macrophage colony stimulating factor bone marrow stromal cell radical oxygen intermediate

5-fluorouracil enhances Egr 1 promoter upregulate expression of granulocyte macrophage colony stimulating factor in human bone marrow stromal cells

Download Fulltext

DU Nan PEI Xue-tao SUN Jun zhong FU Yan ZHAO Hui WANG Xi liang

Department of Oncology, The First Affiliated Hospital of General Hospital of PLA, Beijing 100037, China;Institute of Field Blood Transfusion, Academy of Military Medical Sciences of PLA, Beijing 100037, China;Department of Oncology, The First Affiliated Hospital of General Hospital of PLA, Beijing 100037, China;Department of Oncology, The First Affiliated Hospital of General Hospital of PLA, Beijing 100037, China;Department of Oncology, The First Affiliated Hospital of General Hospital of PLA, Beijing 100037, China;Department of Oncology, The First Affiliated Hospital of General Hospital of PLA, Beijing 100037, China

Fund Project:Surpported by the National Natural Science Foundation of China(N0.39900040); the New Major Technology Program of the First Hospital Affiliated to PLA General Hospital (No.ZD200502)

Abstract:

Abstract Objective: To explore 5 fluorouracil induced regulating effect of Egr 1 promoter on expression of granulocyte macrophage colony stimulating factor (GM-CSF) in human bone marrow stromal cells. Methods:The human GM CSF cDNA and enhanced green fluorescent protein (EGFP) cDNA were linked together with IRES and then inserted into the expression vector pCIneo under control of the Egr 1 promoter(Egr-EG). The vector was then transferred into human bone marrow stromal cell line HFCL by lipofection. The transfected cell clones (HFCL/EG) were selected by the addition of G418. The cells were exposed to the anticancer agent 5-fluorouracil (5-FU). The activity of EGFP in HFCL/EG cells were detected by FACS. The amounts of GM CSF in HFCL/EG postchemotherapy were determined with ELISA. The effects of GM CSF in HFCL/EG cultural supernatants on expansion of CFU-GM derived from cord blood were also studied. The effect of N-acetylcysteine (a free radical scavenger) on GM-CSF production was examined following exposure to 5 FU. Results: We successfully constructed vector Egr EG with Egr-1 promoter, and its transfectant HFCL/EG was obtained. The results indicated that the activity of EGFP and the amounts of secreted GM CSF in HFCL/EG cells exposed to 5-FU were increased compared to non 5-FU group. The content of GM-CSF in HFCL/EG cultural supernatants was significantly higher than that in the non 5-FU group (P<0.01). N-acetylcysteine significantly decreased the content of GM-CSF produced by HFCL/EG treated with 5-FU (P<0.01). Conclusion: 5-FU can enhance Egr-1 upregulate GM-CSF expression in human bone marrow stromal cells, and thus contribute to the recovery of hematopoietic function after chemotherapy.

Keywords:

查看全文 查看/发表评论 下载PDF阅读器