中国医学科学院学报 2007 29 (3): 374-378 ISSN: 1000-503x CN: 11-2237/R

论著

抗凋亡蛋白Bcl-xL在巨核细胞分化和成熟过程中的作用

张 磊, 杨仁池, 卢士红, 刘 斌, 任 贺, 韩之波, 韩忠朝

中国医学科学院 北京协和医学院 血液学研究所 血液病医院 实验血液学国家重点实验室, 天津 300020

收稿日期 2006-6-20 修回日期 网络版发布日期 2007-7-9 接受日期

摘要 摘要：目的 研究抗凋亡蛋白Bcl-x L在巨核细胞分化和成熟过程中的作用。方法 采用PDBu诱导K562细胞向巨核细胞分化,RNA干扰阻断Bcl-x L在K562细胞向巨核细胞诱导分化中的表达,RT-PCR和流式细胞仪技术检测其改变。采用免疫磁珠从正常骨髓中富集CD34+细胞,在无血清培养下用TPO诱导其向巨核细胞分化,免疫组织化学和流式细胞仪技术观察分化过程中Bcl-x L的表达改变。结果 PDBu诱导K562细胞向巨核细胞分化24h后,CD61+细胞百分比迅速增加,并且在72h内维持较高的阳性率;用siBcl-x L干扰后72h内,CD61+细胞百分比只有轻度增加,同时RT-PCR检测显示24h后Bcl-x L mRNA表达显著减少,流式细胞检测显示Bcl-x L蛋白的表达也相应降低。正常骨髓中CD34+细胞经TPO诱导后,在培养5～20d间Bcl-x L蛋白表达阴性的巨核细胞随着培养时间延长逐渐增多,免疫组织化学检测显示未成熟的巨核细胞Bcl-x L蛋白表达呈强阳性,而在成熟的巨核细胞中表达为阴性。结论 抗凋亡蛋白Bcl-x L在巨核细胞分化过程中发挥重要作用,而在巨核细胞发育晚期Bcl-x L蛋白的表达下调可能是巨核细胞成熟的关键,并与成熟巨核细胞的特殊凋亡发生密切相关。

关键词 巨核细胞 凋亡 Bcl-x L 干扰RNA

分类号

Role of Antiapoptotic Bcl-xL in Megakaryocyte Differentiation and Maturation

ZHANG Lei, YANG Ren-chi, LU Shi-hong, LIU Bin, REN He, HAN Zhi-bo, HAN Zhong-chao

State Key Laboratory of Experimental Hematology, Hospital of Blood Disease, Institute of Hematology, CAMS and PUMC, Tianjin 300020, China

Abstract ABSTRACT:Objective To investigate the role of antiapoptotic Bcl-x L protein in megakaryocyte differentiation and maturation. Methods RNA interference was used to block the expression of Bcl-x L when K562 cells were induced to differentiate into megakaryocyte(CD61+ cells) by PDBu, and the expression of Bcl-x L was evaluated with flow cytometry and reverse transcription polymerase chain reaction (RT-PCR). The CD34+ cell fraction was positively isolated by using the MiniMACS system from normal bone marrow. Immunochemical staining and flow cytometry were used to detect the expression of Bcl-x L in the differentiation (CD41+ cells) of CD34+ cells induced by trombopoietin (TPO). Results Among K562 cells induced by PDBu, the percentage of CD61+ cells rapidly increased in 24 hours and maintained at a high positive level in 72 hours. When exposured to si-Bcl-x L, the percentage of CD61+ cells only slightly increased in 72 hours. The expression of Bcl-x L mRNA was significantly decreased after transfection compared with that of control group, and Bcl-x L protein expression decreased correspondingly. After the CD34+ bone marrow cells having been treated with TPO for 5 days to 20 days, the Bcl-x L-megakaryocytes increased as the culture time prolonged, and there was a strong expression of Bcl-x L in immature megakaryocyte and an obviously decreased expression in degenerating mature megakaryocyte. Conclusions Increased expression of antiapoptotic Bcl-x L may be essential to megakaryocytes maturation. The down-regulation of antiapoptotic Bcl-x L in mature megakaryocyte may be crucial to platelets formation.

Key words megakaryocyte apoptosis Bcl-x L siRNA

DOI:

通讯作者 杨仁池 yangrenchi@hotmail.com