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膜结合型干细胞因子促进自血病细胞K562的增殖和集落形成 点此下载全文

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摘要

目的: 探讨膜结合型干细胞因子(membrane bound stem cell factor, m SCF)在白血病细胞中的作用。方法: 克隆并构建携可溶型干细胞因子(soluble st em cell factor, s SCF)前体的真核表达质粒pTARGET s,采用Overlap PCR法去除外显子6序列,进一步构建m SCF的表达质粒pTARGET m,DNA测序鉴定。通过脂质体介导分别将上述载体和空载体pTARGET转染白血病K562细胞,用G418筛选稳定表达细胞株,并用RT PCR、Western blotting法鉴定。通过CCK 8细胞增殖实验以及集落形成实验观察不同细胞株体外增殖特点。结果: 成功构建了s SCF和m SCF的真核表达载体,获得了稳定转染细胞株K562 V、K562 S、K562 M。U底培养条件下,K562 M增殖能力明显高于K562 V和K562 S(均 P <0.01)。K562 M集落形成率显著高于K562 V和K562 S(均 P <0.01),且集落形态大于其他两种细胞。结论: m SCF与C kit之间的并置性作用显著促进白血病细胞的增殖。

关键词: 膜结合型干细胞因子 C kit 白血病 并置性作用

Membrane bound stem cell factor increases proliferation and colony formation of leukemia cell line K562 <u>Download Fulltext</u>

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## Abstract:

Objective: To explore the role of membrane bound stem cell factor (m SCF) in the pathogenesis of leukemia. Methods: The eukaryotic expression plasmid of soluble stem cell factor (s SCF) precursor (pTARGET s) was constructed. Overlap PCR was used to obtain m SCF sequence with the deletion of exon 6, and pTARGET m was constructed. After verified by DNA sequencing, pTARGET m and control pTARGET vector were transfected into K562 cells via Lipofetamine 2000, and the positive cells were screened by G418. K562 cells stably transfected with pTARGET m were verified by RT PCR and Western blotting. Proliferation and colony formation of these stably transfected cells were studied. Results: The s SCF and m SCF eukaryotic expression vectors were successfully constructed. The stable transfectants K562 V, K562 S, and K562 M were obtained. Under U bottom culture condition, proliferation ability of K562 M cells was significantly stronger than those of K562 V or K562 S (both P <0.01). Colony formation ability of K562 M was significantly higher than those of K562 V and K562 S (both P <0.01). Furthermore, the colony size of K562 M was larger than those of the other two kinds of cells. Conclusion: m SCF significantly enhances proliferation and colony formation of leukemia cells by a juxtacrine mechanism.

Keywords: membrane bound stem cell factor (m SCF) C kit leukemia juxtacrine

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