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论著

# mTEL-cFms激酶结构域融合蛋白真核表达载体的构建及其对信号转导和转录激活因子核转位的影响

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**摘要** **目的** 构建激酶盘真核表达载体，观察豆蔻酰化的TEL转录调节因子HLH结构域与c-Fms激酶结构域融合蛋白(mTEL-cFmskd)的表达对信号转导和转录激活因子1(STAT1)和STAT3核转位的影响。**方法** 利用DNA重组技术，将人的c-Src豆蔻酰化多肽、TEL转录调节因子HLH结构域、c-Fms激酶结构域以及c-Myc标签的DNA序列克隆在pCORON/neo载体上，构建pCORON/neo-HcSrc-Tel- cfmskd-Myc真核表达载体。将载体转染至稳定表达GFP-STAT1的人骨肉瘤细胞(U2OS)和稳定表达EGFP-STAT3的幼仓鼠肾细胞24 h后，采用IN Cell Analyzer1000获取细胞图像，分析细胞内GFP-STAT1或EGFP-STAT3融合蛋白的核转位程度。**结果** 质粒酶切和测序鉴定表明，构建的pCORON/neo-HcSrc-Tel-cfmskd-Myc真核表达载体序列正确。载体转染细胞24 h后，绿色荧光蛋白标记的STAT1和STAT3均进入细胞核，发生核转位现象。c-Fms激酶抑制剂GW2580和Sutent能抑制mTEL-cFmskd诱导EGFP-STAT3核转位的发生。**结论** 成功构建激酶盘真核表达载体pCORON/neo-HcSrc-Tel-cfmskd-Myc。载体在细胞中表达的mTEL-cFmskd豆蔻酰化融合蛋白具有M-CSF/c-Fms配体受体复合物激活下游信号分子的生物学功能。

**关键词** 巨噬细胞集落刺激因子受体 真核表达载体 TEL 豆蔻酰化 信号转导和转录激活因子 核转位激酶抑制剂

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# Establishment of mTEL-cFmskd eukaryotic expression vector and its effect on STAT1/3 nuclear translocation

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**Abstract**

**OBJECTIVE** To construct a eukaryotic expression vector of mTEL-cFmskd and study its effect on nuclear translocation of the signal transducer and activator of transcription 1/3(STAT1/3). **METHODS** By recombinant DNA technology, the DNA sequence of polypeptide for myristoylation of human c-Src, helix-loop-helix domain of human TEL, kinase domain of macrophage colony-stimulating factor receptor and c- Myc tag were inserted into pCORON/neo plasmid to generate pCORON/neo HcSrc-Tel-cfmskd-Myc eukaryotic expression vector. The mTEL-cFmskd expression vector pCORON/neo-HcSrc-Tel-cfmskd-Myc and the c-Fms expression vector pCORON/neo-cfms were transfected into U2OS (expressing GFP-STAT1) and BHK (expressing EGFP-STAT3) cells. After 24 h, the cells were fixed, stained and then imaged on the IN Cell Analyzer 1000. The image was analyzed using the Nuclear Trafficking Analysis Module. **RESULTS** Restriction enzyme digestion and plasmid sequencing confirmed the successful construction of pCORON/neo-HcSrc-Tel-cfmskd-Myc plasmid. mTEL-cFmskd was expressed in cells, and caused nuclear translocation of GFP-STAT1 and EGFP STAT3 24 h after transfection. c- Fms inhibitors GW2580 and Sutent could block the nuclear translocation of EGFP-STAT3 by mTEL-cFmskd. **CONCLUSION** mTEL-cFmskd expression vector pCORON/neo-HcSrc-Tel-cfmskd-Myc is successfully constructed and functional mTEL-cFmskd is expressed in GFP-STAT1_U2OS and EGFP-STAT3_BHK cells.

**Key words** macrophage colony-stimulating factor receptor TEL myristoylation STATs nuclear translocation kinase inhibitors

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