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论文

# 艾滋病痴呆综合症患者体内HIV-1 tat基因的克隆及原核表达

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摘要：

目的 探讨艾滋病痴呆综合症（AIDS dementia complex，ADC）患者体内外周和中枢不同组织部位的人类免疫缺陷病毒Ⅰ型（HIV-1）反式激活因子（tat）第一外显子基因序列的变异并对其进行表达，用于研究HIV-1 Tat变异对其神经毒性的影响。方法 从ADC病例尸检标本的脾脏（spleen，SPL）、脑膜（meninges， MG）和基底核（basal ganglia，BG）3个部位的组织中提取基因组DNA，用PCR方法扩增出tat第一外显子基因，并将其插入到pGEM-T克隆载体中，测序证实为HIV-1 tat基因，BioEdit软件对测序结果进行比对。将克隆载体双酶切，回收目的基因，并将其连接到pGEX-KG表达载体中，将重组表达质粒pGEX-KG-tat转化BL21大肠杆菌，异丙基-β-D-硫代半乳糖苷（IPTG）进行诱导表达，表达产物用SDS PAGE和Western blot进行检验和鉴定。结果 成功克隆了HIV-1 tat第一外显子基因，序列比对发现外周和中枢部位的Tat氨基酸序列不同；构建了pGEX KG-tat重组表达载体，并在原核细胞中高效表达了谷胱甘肽巯基转移酶（GST）融合Tat蛋白，SDS PAGE和Western blot分析表明，所表达的蛋白为Tat蛋白。结论 ADC患者外周和中枢不同组织部位的HIV-1 tat第一外显子基因所编码的Tat蛋白氨基酸序列存在变异，在原核细胞中对其进行了高效表达，为Tat蛋白的神经毒性研究奠定了基础。

关键词： 艾滋病痴呆综合症；HIV-1反式激活因子；pGEX KG载体；基因表达

## Cloning and expression of the tat gene from a patient with AIDS dementia complex in Escherichia coli

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Abstract:

Objective To explore variations of the HIV-1 trans-activator of transcription(HIV-1 tat) exon 1 gene from three different tissues of a patient who died of AIDS dementia complex（ADC）and express the Tat-GST protein in Escherichia coli BL21, for investigating the effect of the variation on neurotoxicity of Tat. Methods The tat gene was amplified with PCR from genomic DNA which was extracted from three different tissues, spleen(SPL), meninges(MG) and basal ganglia(BG) of the patient, and the PCR products were cloned into the PGEM-T vector. Sequence results were analyzed with BioEdit. After being cut by BamH Ⅰ-and EcoR Ⅰ and reclaimed, the tat gene was cloned into the pGEX KG vector. The recombinant plasmid pGEX-KG-tat was transferred and expressed in E. coli BL21. Expressed products were identified by SDS-PAGE and Western blot. Results The tat exon 1 gene was successfully cloned. The sequence analysis showed that variations of the HIV-1 tat gene extracted from peripheral and central nerve tissues were different. The recombinant plasmid pGEX-KG-tat was effectively transferred and expressed in E. coli BL21, and SDS-PAGE and Western blot analyses which showed that the expressed protein was Tat. Conclusion The tat exon 1 gene from peripheral and central nerve tissues was cloned and variations exist and the Tat GST fusion protein is efficiently expressed in E.coli, which can contribute to further research on neurotoxicity of Tat.

Keywords: AIDS dementia complex; HIV 1 trans activator of transcription; pGEX KG vector; Gene expression

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