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论著

人IP-10融合蛋白表达载体的构建和表达及活性鉴定

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摘要 目的：构建人His标记的IP-10 (interferon-γ-inducible protein 10)融合蛋白表达载体并在原核细胞中表达、纯化，获得有活性的IP-10蛋白，为进一步研究其在炎症过程中的作用机制及寻找新的抗炎途径提供基础。 方法： 从人肺cDNA文库中PCR扩增无信号肽的IP-10基因编码序列，构建重组载体pET-14b/IP-10；重组质粒经酶切、测序鉴定正确后转化大肠杆菌BL21(DE3)菌株；经异丙基β-D-硫代半乳糖（IPTG）诱导后，用镍离子亲和层析柱纯化融合蛋白，以THP-1细胞进行微室跨膜迁移（transwell）实验鉴定融合蛋白活性。 结果： 酶切、测序鉴定重组载体pET-14b/IP-10构建正确，并纯化得到高纯度的IP-10融合蛋白。而且该蛋白具有诱导单核细胞THP-1跨膜迁移活性。 结论： 成功构建了人IP-10融合蛋白表达载体，并纯化得到具有活性的IP-10融合蛋白，为进一步研究IP-10的功能提供了重要的实验材料。

关键词 γ干扰素诱导蛋白10； 融合蛋白质类； 细胞运动

分类号 Q78

Construction and prokaryotic expression of His-tagged expression vector of human IP-10 and identification of its activity

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Abstract

<FONT face=Verdana>AIM: To construct prokaryotic expression vector of His-tagged human IP-10 for further study of its biological function in the inflammatory response. METHODS: The coding sequence of IP-10 lacking signal peptide was amplified from human lung cDNA library by polymerase chain reaction (PCR) and the fragment was cloned into pET-14b plasmid for the construction of His-tagged fusion protein expressing vector, pET-14b/IP-10. After being identified by enzyme digestion and sequencing, the recombinant vector was transformed into a strain of E. coli, BL21 (DE3). The expression of His-tagged fusion protein was induced with IPTG and purified with Ni+-NTA affinity chromatography. Then the chemotactic activity of IP-10 was determined by transwell migration assay on THP-1 cells. RESULTS: The construction of pET-14b/IP-10 recombinant vector was proved by enzyme digestion and sequencing. The fusion protein IP-10, which was purified by a routine Ni+ affinity method, had an activity on the induction of cell migration of THP-1. CONCLUSION: We successfully construct IP-10 fusion protein expressing vector and get the fusion protein with high bioactivity, which provides essential materials for the future studies on IP-10. V</FONT>

Key words Interferon-γ-inducible protein 10 Fusion proteins Cell movement

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