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论著

重组人CRP的表达纯化及其内化进入HeLa细胞的观察

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摘要 目的： 构建人C-反应蛋白(CRP)原核表达载体，表达纯化his-EGFP-CRP蛋白，观察其能否内化进入HeLa肿瘤细胞。

方法: 利用特异性引物，从p91023/CRP载体上将编码人CRP 的基因序列亚克隆到原核表达载体pET14b/MCS-EGFP-(N) 36上；对阳性克隆进行PCR、酶切和测序鉴定，并将其转化到大肠杆菌BL21(DE3)中诱导表达，表达的重组蛋白通过亲和色谱纯化，梯度透析复性，并与HeLa细胞孵育，利用荧光显微镜观察其内化入胞。

结果: PCR、双酶切和测序鉴定表明，pET14b/EGFP-hCRP原核表达质粒构建正确；转化实验发现，该质粒在BL21(DE3)中能够被大量诱导表达；蛋白纯化及荧光显微镜观察结果表明，复性后的表达产物可结合于HeLa细胞膜，孵育一定时间后，可定位于胞质及胞核。

结论: 成功地构建了带增强型绿色荧光蛋白（EGFP）标签的人CRP原核表达载体，该载体能够在大肠杆菌BL21(DE3)中被诱导表达重组蛋白his-EGFP-CRP，纯化复性后的重组人CRP能与HeLa肿瘤细胞结合，并能内化入胞，移位入核。

关键词 C反应蛋白； 载体构建； 基因表达； 蛋白质复性； HeLa细胞

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Expression and purification of re-constructed human CRP and observation of its internalization into HeLa cells

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Abstract

<FONT face=Verdana>AIM: To construct prokaryotic expression vector for human C-reactive protein (CRP), to acquire the functional fusion protein purified from BL21 (DE3) transformed with vector pET14b/EGFP-hCRP, and to observe the internalization of the fusion protein his-EGFP-CRP into tumor cell line HeLa.
METHODS: CRP gene sequence was amplified with the vector p91023/CRP as template by PCR, and was inserted into vector pET14b/MCS-EGFP-(N) 36 to construct prokaryotic expression vector. The E. coli cells BL21(DE3) transformed with the re-constructed vector pET14b/EGFP-hCRP was induced by isopropyl-β-D-thiogalactopyranoside (IPTG), and the expressed protein his-EGFP-CRP were purified with affinity chromatography method and refolded with gradient filtration. The HeLa cells were observed under the fluorescence microscopy after the addition of purified renature protein.
RESULTS: The results of identification by PCR, digestion with restriction endonuclease and sequencing indicated the construction of vector pET14b/EGFP-hCRP was correct; the SDS-PAGE showed that the transformed E. coli cells could be induced to express the fusion protein his-EGFP-CRP and the purification of proteins were successful. We could found fluorescent signal around the cell membranes, in the cytoplasm and nuclei in the observation of the HeLa cells incubated with his-EGFP-CRP.
CONCLUSION: The prokaryotic expression vector for human CRP linked with his and EGFP coding sequence is successfully constructed. The fusion protein his-EGFP-CRP is purified and refolded. The reconstructed protein expressed by prokaryotic cells adheres to the membrane of tumor cell HeLa and is internalized into the cytoplasm and nuclei of the cells.</FONT>

Key words C-reactive protein Vector construction Gene expression Protein renaturation HeLa

cells

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