

论著

融合蛋白CXCL10-loop3- EGF的可溶性表达及其抗肿瘤效应研究

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摘要 目的: 构建重组趋化因子CXCL10及表皮生长因子受体干扰序列融合蛋白(CXCL10-loop3-EGF), 验证其靶向性抗肿瘤效应及抑制血管形成活性。方法: 运用RT-PCR从经IFN- γ 刺激活化的PBMC中扩增人CXCL10基因, 运用重叠延伸PCR法扩增CXCL10-loop3-EGF融合基因, 并将其与载体pTG19-T连接、转化大肠杆菌DH5 α 、筛选阳性克隆, CXCL10-loop3-EGF融合基因与载体pET32a (+)连接、转化大肠杆菌Origami B(DE3), 诱导可溶性表达重组his-CXCL10- loop3-EGF融合蛋白, 并经镍柱亲和层析、酶切、超滤及透析等方法获得纯化的重组CXCL10- loop3-EGF融合蛋白。通过Transwell细胞趋化实验及HUVEC血管形成抑制实验验证此融合蛋白的抗肿瘤效应。结果: SDS-PAGE和Western blotting证实CXCL10- loop3-EGF融合蛋白构建成功, 纯化后的融合蛋白具有显著的趋化活化PBMC活性及抑制HUVEC血管形成活性。结论: 成功构建融合蛋白CXCL10- loop3-EGF, 该蛋白具有较好的靶向性抗肿瘤活性。

关键词 [CXCL10- loop3-EGF融合蛋白](#) [可溶性表达](#) [抗肿瘤效应](#)

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Soluble expression of a CXCL10-loop3-EGF fusion protein and its anti-tumor activity

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Abstract

AIM: To evaluate the implication of CXCL10-loop3-EGF fusion protein for the activities of targeting tumor and anti-angiopoiesis. METHODS: RT-PCR was performed to amplify CXCL10 coding sequence from PBMC activated by IFN- γ . CXCL10-loop3-EGF fusion gene, which was conducted by Over-Lap Extension PCR, was hinged up with plasmid pTG19-T, transfected to E. coli DH5 α and processed positive colony selection. After ligated with plasmid pET32a(+), recombinant CXCL10-loop3-EGF fusion gene was then transfected to E. coli Origami B (DE3) and induced to express its coding fusion protein his-CXCL10-loop3-EGF. The recombinant fusion protein CXCL10-loop3-EGF was purified by His-bind affinity chromatograph, enterokinase cleavage, ultrafiltration and dialysis. The transwell chemotactic test and HUVEC angiopoiesis inhibition test were performed to determine the anti-tumor responses and anti-angiopoiesis activity of CXCL10-loop3-EGF fusion protein. RESULTS: CXCL10-loop3-EGF fusion protein was successfully constructed and confirmed by SDS-PAGE analysis and Western blotting. Significant PBMC chemotactic activity and HUVEC anti-angiopoiesis activity were observed. CONCLUSION: CXCL10-loop3-EGF fusion protein, which has perfect anti-tumor activity, is successfully constructed.

Key words [CXCL10-loop3-EGF fusion protein](#) [Soluble expression](#) [Antitumor effect](#)

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