

论文

RGD重组人IL-10的克隆、表达及其拮抗纤维化的初步研究

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摘要:

白细胞介素10(IL-10)是一种多效细胞因子,在炎症、免疫反应以及在疾病的发生过程中发挥着重要作用,RGD是能够特异与新生血管内皮细胞整合素结合的多肽序列。将RGD连接到IL-10的羧基端,期望构建新生血管内皮细胞特异导向结合型融合蛋白。以人外周血淋巴细胞cDNA为模板,扩增的PCR产物经克隆载体pMD18-T,连至原核表达载体pET-22b(+),转化大肠杆菌BL21(DE3),构建了pET-IL10-RGD表达载体的重组菌(pET-IL10-RGD/BL21)。SDS-PAGE分析表明:在19.3 kDa处有明显的新生蛋白带,符合理论预期。Western blot分析表明:诱导表达、分离纯化的目的蛋白能够与IL-10抗体特异结合,且纯化产物IL10-RGD具有与IL-10相同的生物学活性。利用培养的人皮肤成纤维细胞,观察了IL10-RGD对TGF-β1刺激成纤维细胞的I型胶原(Col1)、III型胶原(Col3)、α-平滑肌肌动蛋白(α-SMA)、结缔组织生长因子(CTGF)蛋白水平及α-SMA免疫细胞化学的变化。结果表明:纯化产物IL10-RGD能够明显抑制TGF-β1刺激的成纤维细胞Col1、Col3、CTGF和α-SMA蛋白水平的升高;抑制TGF-β1诱导的成纤维细胞向肌成纤维细胞的转化。可见,成功克隆、表达并纯化了IL10-RGD融合蛋白,该融合蛋白能够明显拮抗TGF-β1诱导的纤维化,预示着该蛋白在瘢痕增生及皮肤纤维化治疗方面有着较好的应用前景。

关键词: 白细胞介素-10 新生血管内皮特异结合肽RGD 融合表达 抗纤维化

Cloning, Expression and Analysis of Antifibrosis Function of Fusion Protein Interleukin 10 with RGD

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Abstract:

Interleukin 10 (IL-10) is a pleiotropic cytokine which plays a pivotal role in the inflammatory response, immunological reaction and diseases development. RGD peptide has been demonstrated to bind to integrin on neovasculature endothelial cells specifically. A novel hybrid protein combining RGD with IL-10 was designed. The DNA sequence encoding recombination fusion protein IL10-RGD was subcloned into the pET22b(+) vector for protein expression in E. coli strain BL21(DE3). SDS-PAGE analysis showed an induced expression product band, with a molecular weight of about 19.3kDa, which was consistent with the expected value. The recombinant protein was isolated and purified by Sephadex G25M, and then renatured. Cultured human dermal fibroblasts were treated with transforming growth factor β1 (TGF-β1) stimulation and IL10-RGD treatment. The protein levels of various fibrosis related molecules were assessed by Western blot. The change of α-SMA positive fibroblasts was analyzed by immunocytochemistry. The results showed that IL10-RGD can significantly down-regulate the protein expression levels of Col1, Col3 and α-SMA, and decrease α-SMA positive cells in cultured dermal fibroblasts stimulation with TGF-β1. These findings indicate that IL10-RGD has the antifibrotic effect on dermal fibroblasts, the potential usage in fibrosis diseases by improvement the abnormal deposition of ECM, and suggest that IL10-RGD may be beneficial for the treatment of skin fibrosis and pathological scars.

Keywords: Interleukin 10 RGD peptide Fusion expression Antifibrosis

收稿日期 2011-11-24 修回日期 2011-12-23 网络版发布日期

分类号:

Q789; Q812

基金项目:

扩展功能

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