عنوان مقاله:

Cloning and Expression of Human Keratinocyte Growth Factor in Escherichia coli for Recombinant Drug Production

نویسندگان:

Fatemeh Ebrahimzadeh

Yeganeh Talebkhan

Hassan Mirzahoseini

Ghasem Barati

Massoud Saidijam

خلاصه مقاله:

Background: Keratinocyte growth factor (KGF) is a member of fibroblast growth factor (FGF) family which induces proliferation and differentiation in a wide variety of epithelial tissues. KGF plays an important role in protection, repair of various types of epithelial cells, and re-epithelialization of wounds. Therefore, in patients with hematologic malignancies receiving high doses of chemotherapy and radiotherapy, treatment with KGF decreases the incidence and duration of severe oral mucositis. Objectives: The aim of this study was to express the recombinant form of human keratinocyte growth factor in Escherichia coli. Materials and Methods: KGF gene was amplified by PCR and cloned into the expression vector pET۲۸a(+). The recombinant vectors were transformed into E. coli BL۲۱(DE۳) as expression host and expression of the desired protein was induced by IPTG. The expression was evaluated at RNA and protein levels by reverse transcriptase PCR (RT-PCR) and SDS-PAGE analyses, respectively and the expressed protein was confirmed through western blotting. Results: Cloning was confirmed by PCR and restriction digestion. RT-PCR and SDS-PAGE represented expression of KGF in E. coli. The optimized expression was achieved ۱۶ hours after induction with ۰.۳ mM IPTG at ۳۷°C in luria broth (LB) containing kanamycin. The ۱۸ kDa protein was confirmed by western blotting, using anti-His antibodies. Conclusions: The result of the present study indicated that E. coli expression system was suitable for overexpression of recombinant human KGF and the expressed protein can be considered as a homemade product.

کلمات کلیدی:

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