

عنوان مقاله:

Expression of the Full-length Human Recombinant Keratinocyte Growth Factor in *Pichia pastoris*

محل انتشار:

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خلاصه مقاله:

Keratinocyte Growth Factor (KGF) is a paracrine-acting and epithelium-specific growth factor produced by cells of mesenchymal origin. Based on preclinical data, recombinant KGF plays a critical role in protecting and repairing of damaged epithelial tissues. Despite great efforts to express recombinant human KGF(rhKGF) in different organisms, attempts for finding appropriate protein expression system with the ability of producing a properly folded and processed KGF needs further investigation. *Pichia pastoris* has been used successfully and extensively for production of industrial enzymes and pharmaceutical proteins. Herein, we investigated the affect of pro-region- α -factor early deletion on production and secretion of rhKGF in *Pichia pastoris*. Initially, expression of human KGF induced in MCF-7 cell line treated with 1, 25-Dihydroxy vitamin D3. The coding sequence of full-length rhKGF194 was then cloned into the yeast integrative expression vector, downstream of α -factor and was integrated into *P. pastoris* genome. KGF protein was expressed in *P. pastoris* x33 cells, using α -factor signal peptide for translocation of KGF to ER. An internal human signal peptide was also arranged after α -factor for early removal of the pro-region in ER. RT-PCR results demonstrated that KGF mRNA was expressed successfully after induction by methanol. Recombinant KGF protein expression was detected by Western blotting in cell lysates, but not in conditioned media. A molecular weight of 17 kD for rhKGF194 indicates that the α -factor and internal human signal peptide had been removed in x33 cells. The results indicate that in the absence of pro-region- α -factor, the recombinant KGF protein was not efficiently processed and transported within the biosynthesis-secretory pathway. As KGF protein is an unstable growth factor and tend to aggregate because of some native properties, It seems that presence of a chaperon molecule fusion with KGF is necessary for efficient secretion of the recombinant protein.

کلمات کلیدی:

Keratinocyte growth factor, *Pichia pastoris*, Signal peptide

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