Cloning and expression of pdgf-bb gene in eschrichia coli

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Background and Aims: Platelet-derived growth factor (PDGF) is an important mediator in wound healing. PDGF is a potent mitogen for fibroblasts and other cells of mesenchymal origin. It is chemotactic for neutrophils, monocytes, fibroblasts, and smooth muscle cells. PDGF stimulates production of several connective tissue molecules and supports angiogenesis. A gel formulation of recombinant human PDGF (rhPDGF) is currently approved to treat full-thickness ulcers in diabetic patients.

Methods: Coding sequence of PDGF-BB gene after optimization was synthesized, PDGF-BB gene and PET15b vector were digested with restriction enzymes. The gel-purified digested PDGF-B gene was cloned into pET15b. Successful construction of the pET15b-PDGF-B clone was confirmed by restriction digestion and PCR amplification. For protein production, host E. coli strain BL21(DE3) was transformed by insertion of a recombinant PDGF-B plasmid. After growth and harvesting of E. coli BL21(DE3)/PET15-hPDGF-B in Luria-Bertani (LB) medium containing 100 µg ampicillin/ml at 37°C until the OD600 reached 0.6, after which 1 mM IPTG was added and culture continued for another 4 h. Analysis of the expression samples was carried out using SDS-PAGE method.

Results: After cell culture and induction of expression with IPTG, SDS-PAGE was done & The gel shows increasing expression of gene product following induction with IPTG.

Conclusions: The construction of recombinant prokaryotic expression vector pET-PDGF-BB and the preparation of PDGF-BB protein provide a foundation for further study of the function of PDGF-BB and producing biological PDGF-BB Protein.

Keywords: Cloning; PDGF-BB; E. coli