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Cloning of the human BDNF (Brain-derived neurotrophic factor) gene and functional expression

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Introduction

Brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor (NGF) gene family, has been shown to influence the survival and differentiation of specific classes of neurons in vitro and in vivo. To observe functional expression of BDNF gene, we are cloning human BDNF gene first of all. We using prokaryotic overexpression system, eukaryotic system. We describe an aspect that BDNF gene overexpression in *Escherichia Coli*. Porkaryotic overexpression system does not have cellular localization signals that may affect posttranslational modifications and the active state of the BDNF gene. The synthesized peptide is biologically inactive. We investigate valuable *Chlorella* as a new bioreactor for mass production of useful target proteins. We construct transformed *Chlorella* and assay.

Materials and Methods

HUMAN BDNF GENE CLONING : Human BDNF gene was cloned by RT/PCR. RT/PCR reaction template was human brain hippocampus total RNA (bioneer) and used specific primer. Annealing temperature process 48℃ .

CONSTRUCTION OF CLONING VECTOR : By using pGEM-T cloning vector, performed ligation and transformation in XL1-Blue *E.Coli* host strain.

SCREENING OF HUMAN BDNF AND SEQUENCING ANALYSIS : Isolated human BDNF clone was reacted with ABI PRISM Dye reagent (Perkin Elmer) and sequenced with ABI 310 Genetic Analyzer (Perkin Elmer).

EXPRESSION SYSTEM : We used medaka (*Oryzias latipes*) eukaryotic system, Micro algae *Chlorella ellipoidea* eukaryotic system and BL21(DE3) *E.Coli* prokaryotic

system.

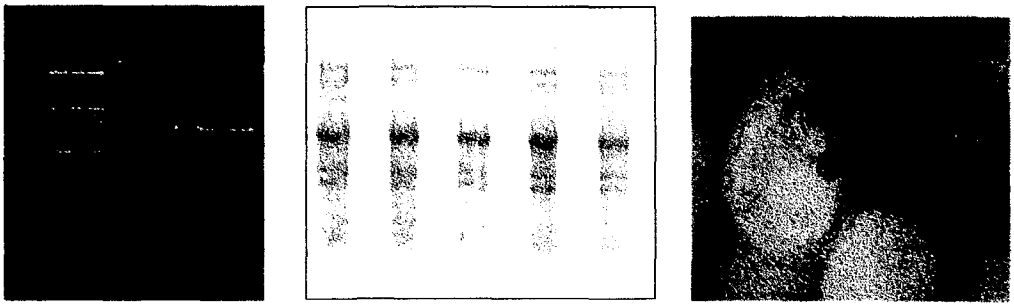
OVEREXPRESSION IN PROKARYOTIC SYSTEM AND WESTERN BLOTTING : By using pGEX-4T-2 (GST fusion) vector, performed ligation with BDNF gene and transformation in BL21(DE3) *E.Coli* strain. In order to overexpression of BDNF gene, we treated 0.1mM IPTG in transformed *E.Coli*.

TRANSGENIC MEDAKA BY ELECTROPORATION : Medaka embryos was rinsed in PBS/-Ca++ and placed in a 0.4cm wide cuvette with 800$\mu\ell$ of the DNA solution. Concentration of DNA approximately 100μg/ml. The embryos was pulsed three times using 0.25μF capacitor of the Gene Pulser apparatus (Bio-Rad). The pulse filed strength is 125/cm. For monitoring medaka embryo development, we observed in fluorescence microscope.

PROCESSING OF *Chlorella* TRANSFORMATION : After *Chlorella* protoplast formation, 5μg mpCTV expression vector ligased BDNF was added with 25μg calf thymus DNA as carrier and carried out transformation. The transformed cells were transferred to fresh f/2 medium and cultured.

Result and Discussion

The neucleotide sequence of human BDNF has shown an ORF consisting 744bp, which has 248 amino acids. Molecular weight expressed GST-BDNF fusion pretein is about 57KD.



(A) (B) (C)

Figure (A) Human BDNF gene clone by RT/PCR processing. 744bp detection in 1.0% agarose gel electrophoresis. (B) Overexpression of BDNF gene in E.Coli. (C) Transgenic Medaka by Electroporation.

References

Martin Korte, P.C. ,Eckhard W, Gottfried Brem, H. T. and Toblas B.(1995) Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. pp.8856-8860,Vol.92, Neurobiology

Anne E. West, Wen G. Chen, M. B. Dalva, Ricardo E. Dolmetsch, Jon M. Kornhauser, A. J. Shaywitz, M. A. Takasu, Xu Tao, and M. E. Greenberg (2001) Calcium regulation of neuronal gene expression. pp 11024-11031 vol. 98 PNAS.