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Molecular studies on the BDNF (Brain-derived neurotrophic factor) from flounder (*Paralichthys olivaceus*)

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Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that also includes NGF, NT-3, NT-4/5, NT-6 and NT-7 (Hashimoto and Heinrich, 1997). The neurotrophins have been extensively studied in birds and mammals. These studies revealed that neurotrophins play critical roles in the regulation of neuron survival during periods of natural cell death.

Materials and Methods

Construction of flounder pituitary cDNA library

A pituitary cDNA library was constructed from pituitaries that were obtained from male and female flounder at all reproductive stages. cDNA library was constructed as described in the Manufacturer's instruction (Stratagene, cDNA library construction kit).

Purification of plasmid DNA and Sequence analysis

The clone obtained from screening of library was purified using Wizard Plus SV minipreps DNA purification system (Promega) and checked for the size of the inserted cDNA. The nucleotide sequences of cloned cDNAs were determined by a chain-termination method using BigDye terminator premix kit (Perkin Elmer). Sequencing was carried out using T3 and T7 Universal primers. The fluorescence-labeled nucleotides were analyzed on an ABI PRISM™ 310 automatic sequencer (Perkin Elmer).

Results and Conclusion

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1      GCACGAGGTT ACGTATATGT GTTCAAGCTC CTTTCTGACA ACGCAGCCTT
51     GTTGATAGTT GTCTCCTTAC TGTAGAGCCG CCGAGGTGTT GCCTGCACTG
101    AGCAGAGTTT TAAGCACGGC CGATATTGCA AAGGGTTATT AGATTCATAA
151    GTCAGGCGAA GTGGTGGCGC ATCTACTGAG CACAGCAGAA GTTCTCGTAT
201    GATGACTTCA AACCAAGACAC ATTACCTACC AGCATCTGTT GGAGTGAGTG
251    GATATTAAGA CACTTGTATC TCCAGGACAG CGCAGGGGGA AAATGTTCCA

301    CCAGGTTAGA AGAGTGATGA CCATCCTGTT CCTTACTATG GTTATTTTAT
351    ACTTCAGTTG CATGAGAGCT GCGCCCTGTA GAGACGCCCC GGGCATGCGG
401    GGCCATCGGA CGGAAGGCTA CTTGGGCGCT GCTGCGACGG CCGCAAGAGG
451    CCATGGGACT CCACAGAGTG GTGGTGGACC AGGCCAGCGT GGGGAAC TGC
501    CCTCGCTCGC AGACACGTTT GAGCAAGTGA TAGAGGAGCT GCTGGAGGTG
551    GAGGGAGAGG CGGCACAGCT GGGACAGGGG GCTGATAAGA GCCAGGGAGG
601    TGGGGGCCCA TCCTCTGTGG TCAACGCAGA GAGCAAGGAT GTCGACCTGT
651    ACGACTCACG GGTGATGATC AGCAACCAAG TGCCTTTGGA GCCGCCCTTG
701    CTCTTTCTCC TGGAGGAATA CAAAACTAT CTGGACGCAG CTAACATGTC
751    CATGAGGGTG CGGCGACACT CCGATCCCTC ACGGCGTGGG GAGCTCAGTG
801    TGTGTGACAG TATTAGCCAG TGGGTGACAG CTGTGGATAA AAAGACGGCA
851    ATAGACATGT CTGGGCAGAC AGTTACCCTC ATGGAAAAGG TCCCTGTCCC
901    CAATGGCCAA CTGAAGCAAT ACTTTTATGA GACCAATGC AACCCCATGG
951    GGTACACAAA GGAGGGCTGC AGAGGAATAG ACAAGCGGCA TTATAATTCC
1001   CAATGCAGGA CAACCCAGTC CTACGTGCGA GCGCTTACCA TGGATAGCAA
1051   AAAGGAGATT GGCTGGCGGT TTATAAGGAT AGACACTTCA TGTGTATGCA
1101   CATTGACCAT TAAAAGAGGG AGATAGTGTA TAAAATGTAT AGATTTTATT

1151   GAAGAGTTTA AAAAAGAGAA TGAAGAGAAA AGATCTATTT GTATATATAC
1201   ATAACAGGGT AAATTATTCC GTCAAATGAA AATTTTATGG ACTGCATGTA
1251   AAAAAGATG AAGTTTATAC AGTAAAAGTG ATACTACAGT CTATTTATTTG
1301   AACATATTCA TGACCTTGTA AACCAATTAA AAAAATCTGA TCAGTCATTT
1351   GCGCCAGTT TAAATTACTA TATCACAATC CTCAGACAT TGTGATTTGT
1401   TTACGTTGCC AAGAATTAGA AAAAAA AAAA

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Fig 1. Sequence of flounder BDNF gene

The gene encoding flounder BDNF was cloned from a flounder brain cDNA library. The probes used for screening the library were cloned cDNA fragments, generated by PCR amplification of cDNA from brain cDNA phage library. The nucleotide sequence of BDNF has shown an open reading frame (ORF) consisting 810 bp that corresponds to a protein, which has 269 amino acids and one stop codon. The nt and peptide sequences were compared to those of other BDNF genes from other species. It showed as high as 93% in southern platy fish. We also constructed a phylogenetic tree base on BDNF amino acid sequences from various species.

References

Hashimoto, M. and Heinrich, G. 1997. Brain-derived neurotrophic factor gene expression in the developing zebrafish. *Int. J. Devl Neuroscience*. 15(8). 983-997.