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Cloning of Rat BDNF and Construction of Recombinant Targeted Expression Vector pGPAF

Hou Ling-ling, Xia Zong-xin, Li Fei and Xing Ying*

Department of Neurology, China-Japan Union Hospital of Jilin University, Changchun - 130033, China.

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Brain-derived neurotrophic factor is one of the most major neurotrophic factors in brain, there are a variety of biological functions such as nerve protection, restoration and prompting neurons,and so on. Due to the less content in brain tissue and exogenous BDNF can not through the blood brain barrier,its applications are limited. In order to research the feasibility of expression vector carrying BDNF through the blood-brain barrier, this study firstly built a targeted expression vector pGPAF - BDNF. from the NCBI ,we got the gene sequence of rat BDNF.with codon optimization, artificially synthesized rat BDNF full genetic sequence. after recovery, purification, enzyme digestion and reforming,it is connected to the pCMV carrier and the pcDNA pGPAF carrier, the recombinant plasmid pCMV - BDNF and pGPAF – BDNF transform into *E.coli* DH5α, screening positive clones and enzyme identification. the results show that we successfully clone the BDNF gene and build a targeted expression vector pGPAF – BDNF.

Key words: Brain-derived neurotrophic factor, Clone, Expression vector, Targeted, Construction.

Brain-derived neurotrophic factor(BDNF) is a protein with neurotrophic effect first found in pig head by Barde in 1982[1] BDNF and its receptors are widely expressed in nervous system,such as the central nervous system and peripheral nervous system, endocrine system and bone and cartilage tissue, but they are mainly expressed in the central nervous system, especially hippocampus and cortex[2]. BDNF is one of the most major neurotrophic factor in the brain, which maintain and promote the growth of nerve cells, in recent years many studies have shown that it has a positive role in protection and restoration during the brain ischemia injury[3-4]. Under normal conditions, endogenous BDNF is very low, and exogenous BDNF is not easy through the blood brain barrier with its big molecular weight , the studies of BDNF in the central nervous system is affected. Current gene therapy of intracerebral diseases mostly adopts the virus as a vector.

Through craniotomy lateral cerebral ventricle injection or around lesions injecting is often and through peripheral injection way is less. However viral vectors have toxicity and vice injury problems to restrict its applications , Looking for a safe and effective treatment is a hot spot in current research. For this ,we obtained BDNF gene sequence by PCR amplification, after recovery, purification and macrorestriction connected to pCMV and pGPAF carrier, aims to study the feasibility of non-viral vector carrying BDNF penetrating blood brain barrier and provide an experimental basis for the future.

* To whom all correspondence should be addressed
Xing Ying
Tel.: 13844021603; Fax: 86-0431-84995820;
E-mail: xingying1970@163.com;

MATERIALS AND METHODS

Prokaryotic expression vector (purchased from GE company), the cells DH5α^5 kept by ourselves, the genome extraction is from the human blood; DNA gel recovery kits , a small plasmid extraction kit, EcoRI restriction endonuclease BamHI restriction endonuclease, Marker DL2000(tiangen biochemical science and technology,Beijing), BDNF primer sequence synthesis and whole sequence synthesis by Shanghai ShengGong company to complete. Reference to GenBank rat BDNF gene sequence, by Primer5.0 software design upstream and downstream primers sequences respectively, the upstream primer sequence is as follows: F 5'-cgc gga tcc atg acc atc ctt ttc ctt act atg gtt att tc -3', the downstream Primer sequences: R 5'-gc gaa ttc cta agg aag tgt caa tac gaa tga-3'. GPAF upstream primer: 5 '--ga aga tct agg aaa ggt ctg agg gtt g-3', GPAF downstream primer: 5'-gg atg cat gga agg tgg gtc aag aaa3'.

The rat BDNF gene sequence optimization and synthesis

Getting BDNF by NCBI gene sequence (GI: NM - 001270630.1), using bioinformatics software, according to the codon bias of e. coli on the sequence optimization, end with BamHI and EcoRI respectively before and after the enzyme loci, and synthetic by Shanghai ShengGong company .

Rat BDNF gene sequence amplification and construction of pCMV - BDNF expression vector

PCR amplification with synthetic sequence , reaction of 50 ul , reaction conditions as follows: 94°C degeneration 3 min, 94 °C modified 30 s , 58°C annealing 50s, 72 °C extension for 1 min, a total of 30 cycles, the last 72 °C extensions for 7 min, reaction products by 1% agarose gel electrophoresis identification. after identifying the correct recycle and purifying of PCR products with BamHI and EcoRI restriction enzymes double enzyme digestion, enzyme product connection after recovery and purification in the BamHI and EcoRI restriction enzymes enzyme pCMV carrier, and translated into competent escherichia coli DH5 alpha cells. By microbial PCR identification of positive clones to Beijing golden extreme intelligence sequencing biological company.

The transformation of pcDNA carrier and the construction of pGPAF - BDNF expression vector

According to the carrier pcDNA mapÿcut carrier original CMV promoter by BglII and XholI double enzyme and join GPAF promoter, identify wth PCR and enzyme digestion. After modification of the carrier by BamHI and EcoRI restriction enzymes, insert segments of BDNF, and translated into competent *Escherichia coli* DH5α cells. The microbial PCR identification for positive clones and Sequencing.

RESULTS

Rat BDNF gene PCR amplification results

With synthetic sequence as the template, F, R for upstream and downstream primers, PCR amplification, amplification product has been confirmed as the purpose of gene frag -ment,which is closed to 714 bp by 1% agarose gel electrophoresis detection (Fig. 1).

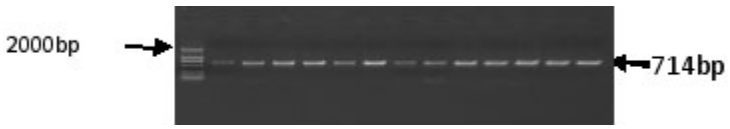

Fig. 1. The results of rat BDNF gene PCR

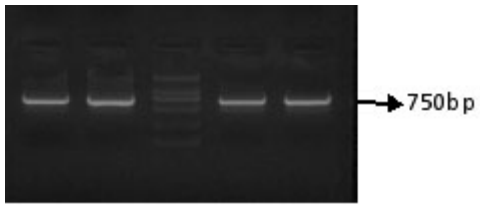

Fig. 2. Microbial PCR amplification results of the rat BDNF gene

Building and sequencing of pCMV -BDNF expression vector

With BamHI and EcoRI restriction enzyme, BDNF and pCMV carrier are recovery and purified, T4 ligase connection into *E. coli* DH5α cells, positive clones of microbial PCR identification were sent to Beijing golden extreme intelligence company sequencing, by 1% agarose gel electrophoresis electrophoresis,microbial PCR amplified result show that the electrophoresis of 750 bp has specific bands (Fig. 2), as same as expectations, positive cloning sequencing shows correct. (Fig. 3).

Modifying of pcDNA carrier and construction of pGPAF -BDNF expression vector

With the extracting genome as a template for GPAF sequence amplification, amplification product is detected by 1% agarose gel electrophoresis , there are specific bands with 2000 bp in the electrophoresis, closing to the expected 2000 bp, which is for the target gene fragment (Fig. 4). After BglII and XholI restriction enzymes digestion, GPAF amplification fragment is connected to the pcDNA carrier with T4 ligase connecting into e. coli DH5 alpha cells, positive

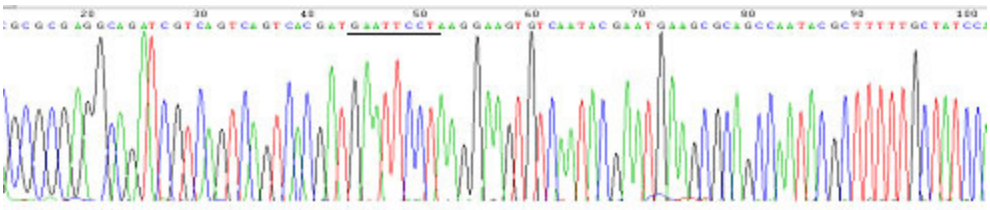

Fig. 3. Part sequencing results of rat BDNF gene

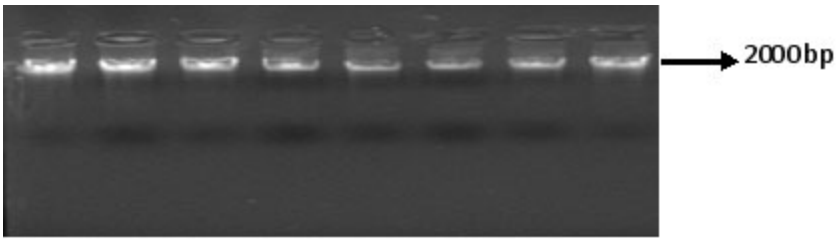

Fig. 4. PCR amplification results of GPAF gene fragment

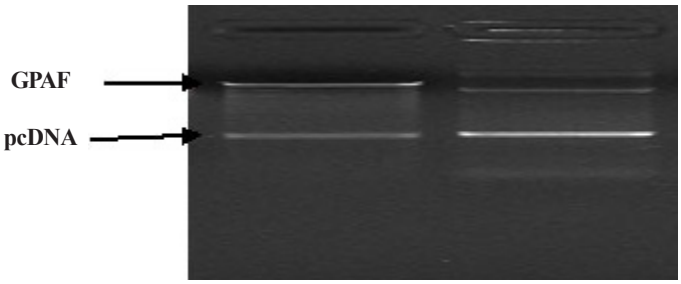

Fig. 5. pcDNA carrier enzyme identification results

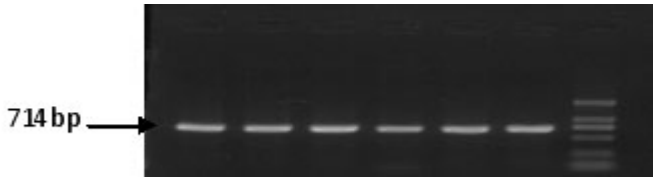

Fig. 6. Microbial PCR amplification results of the rat BDNF

clones of the microbial of PCR identification is for enzyme identification (Fig. 5). After BamHI and EcoRI restriction enzymes digestion , the identified correct carrier is connected with the fragement of BDNF and T4 ligase which transform into e. coli DH5a cells, positive clones by microbial PCR identification are sequenced, with 1% agarose gel electrophoresis electrophoresis, microbial PCR amplified result has specific bands in the electrophoresis of 750 bp (Fig. 6), as same as expectations, positive cloning sequencing shows correct. (Fig. 7)

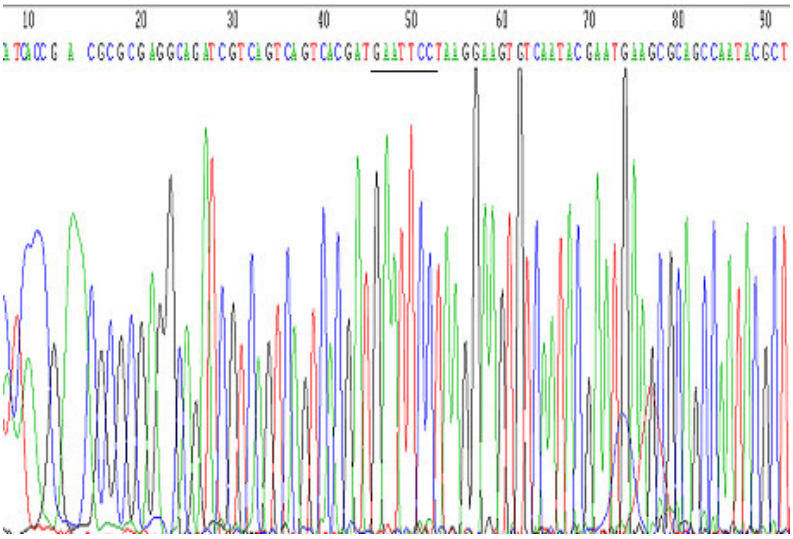

Fig. 7. Part of pGPAF-BDNF sequencing results

DISCUSSION

BDNF is a member of the neurenergen family, it plays an imprtant role in many aspects,which promote the growth and differentiation of neurons in the central nervous system. due to the presence of the blood-brain barrier, it is difficult for exogenous BDNF to enter BBB with such macromolecular , and secretion of endogenous BDNF is few, is not enough to play a role of biology. The current application of BDNF gene as a means of treatment mostly adopt direct injection or lateral ventricle injection with virus as the carrier, but its side effects limit the wide application of [6]. In recent years, some scholars try to make liposomes as a non viral vector carrying genes to penetrate the blood-brain barrier[7]. But the low rate of encapsulation efficiency limits its development. This study adopts the method of genetic engineering to get the seqence of BDNF from the NCBI ,by codon optimization, artificial synthesize BDNF full genetic sequence.after recovery, purification and enzyme digestion to connect to the pCMV carrier and the pcDNA pGPAF carrier. after reforming, the recombinant plasmid pCMV - BDNF and pGPAF - BDNF transform into e. coli DH5a cells, screening positive clones and enzyme identification.

GPAF promoter can only start the specific target gene expression in cerebral neurons and astrocytes, modified pGPAF - BDNF have higher targeting , can effectively reduce the expression of desired genes in the surrounding organs, the study changes the original pcDNA carrier to GPAF promoter, in order to increase intracranial BDNF levels to the greatest extent and play its protective role better.

This study successfully builds two kinds of expression vector both pCMV - BDNF and pGPAF - BDNF by biological engineering technique, by colony PCR and gene sequencing confirmed that the homology of cloned fragment and optimization fragment was 100%, this is experimental basis for the next research that fetures and related function of non viral vectors with BDNF penetrating blood brain barrier .

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