Construction of Prokaryotic Expression Vector of BRD7 and Its Expression in *E. coli* *

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Abstract BRD7 gene is a good candidate tumor suppression gene associated with NPC. In order to construct prokaryotic expression vector of BRD7 and express BRD7 in *E. coli*, The coding region with *Sal* I and *Not* I restriction sites of BRD7 was obtained from pGEM-T Easy/BRD7 plasmid by PCR. PCR product and plasmid PGEX-4T-2 were digested by corresponding restrict endonucleases respectively. The fragments were ligated by T4 DNA ligase to gain recombinant expression vector. Endonuclease digesting and DNA sequencing confirmed that the coding region of BRD7 gene was correctly inserted into the vector. The recombinant plasmid PGEX-4T-2/BRD7 was transferred into competent Jm105 strain. The GST/BRD7 fusion protein was expressed in the bacteria under induction of IPTG. After induction, a new protein band of 90 ku appeared on SDS-PAGE. The result was confirmed by Western blot. The recombinant protein of 90 ku amounted to 28.48% of the total bacterial protein after inducing with IPTG for 4 h at 37 ℃. It existed not only in supernatant but also in precipitation of broken bacteria. The successes in construction of expression vector of BRD7 and expression of BRD7 in *E. coli* make it possible to study further on its biological function and antibody preparation.

Key words nasopharyngeal neoplasms, gene expression, BRD7 gene

Nasopharyngeal carcinoma (NPC) is a tumor with a striking geographical and ethic distribution. Epstein-Barr virus (EBV), genetic predisposition, dietary and environmental factors are all believed to play a role in the development of this tumor[1,2].

The statistic analysis revealed that 10% ~ 15% of NPC patients have family history and that Chinese in Southern China and emigrant Chinese in other countries have a high NPC incidence rate[3]. Therefore, genetic susceptibility might play an important role in the initiation and progression of NPC.

We examined expression of cDNA fragments isolated by cDNA representational difference analysis (cDNA RDA) in NPC biopsies, and cloned a downregulated gene BRD7 (GenBank Accession No. AF179285) associated with NPC. Transgenic experiment showed that BRD7 present obviously suppressive role on NPC cell growth[4]. Further study on cellular sublocalization of green fluorescent protein (GFP) tagged BRD7 revealed dual localization both in nucleus and in the cytoplasm[5]. In addition, we also found that, of the three cSNP (coding region single nucleotide polymorphisms) of BRD7 gene detected, T450 and G737 might be one of important risk factors for the development and/or progression of NPC ($P < 0.01$)[6]. Furthermore, we had used a two-hybrid screen in yeast to identify proteins that interact with BRD7. These data implied that BRD7 might be a good candidate of tumor suppressor gene associated with NPC. Thus, expression of BRD7 and antibody preparation of it bring us a new clue for researching on molecular genetic mechanisms of NPC, and give us a promising area in the preventment and treatment of NPC.

1 Materials and Methods

1.1 Materials

1.1.1 Plasmid and strain. pGEM-T Easy/BRD7 plasmid was constructed by our laboratory, which includes the full-length cDNA of BRD7 gene. PGEX-4T-2 [a glutathione-S-transferase (GST) fusion protein vector] was provided by Pharmacia Biotech. Strain Jm105 was conserved by our laboratory.

1.1.2 Enzymes and kits. Restriction endonucleases *Sal* I, *Not* I and T4 DNA ligase, Taq DNA polymerase were obtained from Promega Company. Gel extraction kit, PCR purificaton kit and plasmid extraction kits were purchased from Shanghai Waston Biotechnologies Inc.

1.2 Methods

1.2.1 Amplification of the BRD7 gene. The full-length cDNA of BRD7 gene was amplified from plasmid pGEM-T Easy/BRD7 by PCR. Primer used for PCR corresponding to the coding region of the BRD7 gene are 5′-AAGTCGACAATGGGCAAGAA-GCACACAAGA-3′ and 5′-TAGCGGCCGCTCAAC-TTCCACCAGGTG-3′ (The underlines show the restriction sites of *Sal* I and *Not* I). The PCR amplification was performed for 30 cycles of 94 ℃ for

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50 s, 57 ℃ for 50 s and 72 ℃ for 1 min.

1.2.2 Construction and identification of the PGEX-4T-2 /BRD7 recombinant expression vector. Both the BRD7 gene and PGEX-4T-2 plasmid were digested with *Sal* I and *Not* I. Then, the BRD7 gene was inserted into PGEX-4T-2 vector at the restriction endonuclease sites by the use of T4 DNA ligase. The recombinant plasmid was identified by sequencing and digesting with *Sal* I and *Not* I.

1.2.3 Expression of BRD7 gene in *E. coli*. The PGEX-4T-2/BRD7 plasmid was used to transform *E. coli* Jm105 competent cells. Sterile 2-YT broth was inoculated with an overnight culture of transformed *E. coli* at a dilution of 1/100, and incubated at 37 ℃ with shaking. When the absorbance at 550 nm reached 0.5, GST-BRD7 expression was induced by the addition of 1 mmol/L isopropyl-β-D-thiogalactoside (IPTG). 3~5 h after induction, the cells were pelleted by centrifugation at 10 000 *g* for 5 min and resuspended in 10 ml of lysis bufffer (1 mol/L Tris-HCl, 0.5 mol/L EDTA, 0.5% Triton X-100). The resuspended cell were disrupted by sonication at 50 W, and centrifuged at 5 000 *g* for 15 min. The supernatant was retained for SDS-PAGE electrophoresis.

1.2.4 Western blot analysis. Twenty five micrograms of protein from *E. coli* cell extracts was resolved by SDS-PAGE and transferred to Biotrac nitrocellulose by using a Trans-blot cell apparatus according to the manufacture's introductions. Filter were incubated sequtially with anti-GST polyantibody (1∶1 000 dilution) for 1 h at 37 ℃, then incubated with goat-anti-rabbit IgG alkaline phosphatase conjugate. The protein was visualized by the addition of substrate as described by the supplier (Bio-Rad laboratories).

2 Results

2.1 Amplification of the BRD7 gene

Following amplification by PCR, the product was examined by agarose gel electrophoresis (Figure 1) for the presence of the BRD7 gene. A band of approximately 2 000 nucleotides was observed and identified.

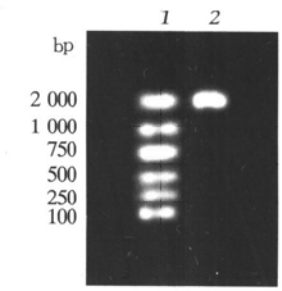

Fig. 1 Agarose gel electrophoresis of the amplification of BRD7 from plasmid pGEM-T Easy/BRD7

1: molecular mass marker; *2*: amplified product of BRD7.

2.2 Construction and identification of the pGEX-4T-2/BRD7 recombinant expression vector

The recombinant plasmid PGEX-4T-2/BRD7 was identified by the digestion with restriction endonuclease *Sal* I and *Not* I (Figure 2). The result shows that it had been divided into two bands. One is the PGEX-4T-2 plasmid (4.9 kb), the other is BRD7 gene (2 kb). The result was also confirmed by sequencing (Figure 3). The nucleotide sequence of the PGEX-4T-2/BRD7 confirmed the BRD7 gene had been inserted into PGEX-4T-2 plasmid and the inserts were in-frame with GST, so the recombinant plasmid is constructed correctly.

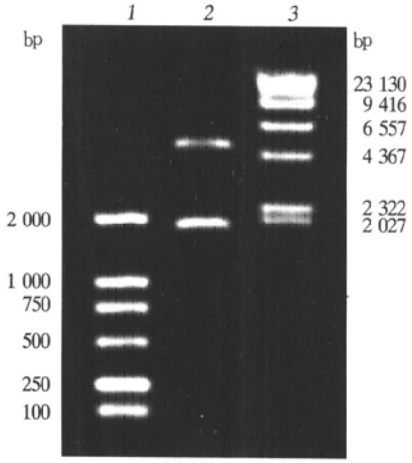

Fig. 2 Identification of recombinant plasmid PGEX-4T-2/BRD7 by digesting with *Sal* I and *Not* I

1: molecular mass marker 1; *2*: the product of plasmid PGEX-4T-2/BRD7 digested with *Sal* I and *Not* I; *3*: molecular mass marker 2.

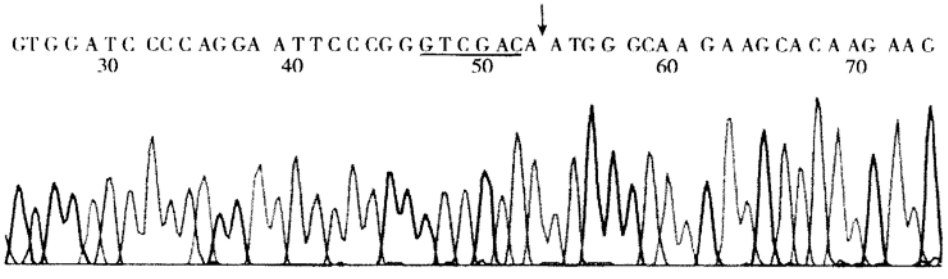

Fig. 3 Sequence of the PGEX-4T-2/BRD7 plasmid

The underlines show the restriction site of *Sal* I, the arrow indicate the initiation codon of BRD7.

2.3 Expression of BRD7 gene in *E. coli*

The recombinant BRD7 gene was successfully expressed in a soluble form, as a fusion protein with GST. The electrophoretic mobility of the expressed protein approximated to a molecular size of the 72 ku BRD7 plus the 26 ku GST (Figure 4), and constituted 28.48% of the total protein after induced with IPTG for 4 h at 37 ℃.

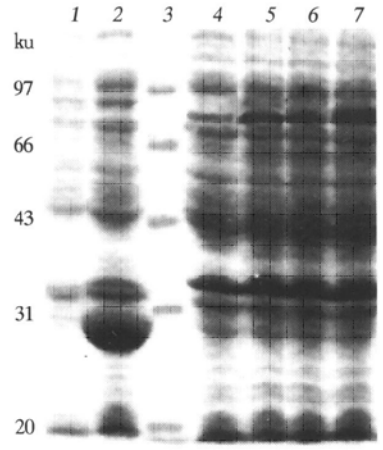

Fig. 4 SDS-PAGE analysis of expression of GST-BRD7 fusion protein

1: uninduced GST protein; *2*: induced with IPTG for 4 h GST protein; *3*: protein molecular mass marker; *4*: uninduced GST/BRD7 protein; *5*: induced with IPTG for 3 h GST/BRD7 protein; *6*: induced with IPTG for 4 h GST/BRD7 protein; *7*: induced with IPTG for 5 h GST/BRD7 protein.

2.4 Western blot analysis

Western blot analysis showed that a new anticipated protein of GST/BRD7 appeared apparently (Figure 5).

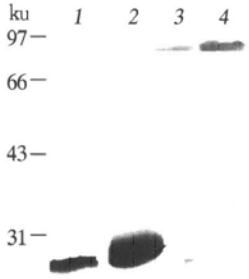

Fig. 5 Western blot analysis of GST/BRD7 fusion protein

1: uninduced GST protein; *2*: induced with IPTG for 4 h GST protein; *3*: uninduced GST/BRD7 protein; *4*: induced with IPTG for 4 h GST/BRD7 fusion protein.

3 Discussion

Precious studies on genetic changes in NPC demonstrated that known oncogenes and tumor suppressor genes, such as *c-myc*, P53 and P16, might be involved in tumorigenesis of NPC[7]. More and more data showed that there are other potential genes involved in pathogenesis of NPC. Endeavors are focusing on researching for the genetic alterations in NPC and isolations and identification of novel genes associated with NPC. Therefore, it can lead to better understanding of molecular basis of NPC, and it will also be helpful in designing improved strategies for prevention, diagnosis and therapy of this cancer. BRD7 gene is one of 18 genes related with NPC which are cloned by our laboratory. A series of experiment showed BRD7 gene is a good candidate tumor suppressor gene related with NPC, so the protein expression and antibody preparation laid a solid foundation for further research about the biological function of BRD7.

The PGEX-4T-2 plasmid is designed for inducible, high-level intracellular expression of genes or gene fragments as fusions with *Schistoma japonicum* GST. GST fusion protein is produced in *E. coli* cells carrying a recombinant PGEX plasmid. Protein expression from a PGEX plasmid under the control of the tac promoter, which is induced using the lactose analog isopropyll-D-thioglctoside(IPTG). Yield of fusion protein is highly variable and affected by the nature of the fusion protein, the host cell and the culture conditions used[8]. During the expression of BRD7 in *E. coli*, we investigated many vectors and strains, PGEX-4T-2 and Jm105 were finally chose as the plasmid and host bacterial strain for the expression of the BRD7 gene. In addition, we also optimized expression conditions, induced with IPTG for 3~5 h at 37 ℃ was selected finally. At present, we are readying to purify the fusion protein for the production of the polyantibody of BRD7.

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鼻咽癌侯选抑瘤基因 BRD7 原核表达载体的构建及其表达*

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摘要 BRD7 基因是一个鼻咽癌侯选抑瘤基因, 为了构建 BRD7 基因的原核表达载体并使其在大肠杆菌得到表达, 设计了带有 *Sal* Ⅰ, *Not* Ⅰ 酶切位点的引物, 以已构建好的质粒 pGEM-T Easy/ BRD7 为模板, 用 PCR 扩增出 BRD7 基因的完整阅读框架, 并用 *Sal* Ⅰ, *Not* Ⅰ 酶切 PCR 产物和原核表达载体 PGEX-4T-2, 然后用 T4 DNA 连接酶将其连接, 得到重组表达质粒 PGEX-4T-2/ BRD7, 经双酶切鉴定和测序验证, 表达载体构建正确. 重组表达质粒转化感受态大肠杆菌 Jm105 后用 IPTG 诱导, 成功表达了一分子质量约为 90 ku 的融合蛋白; 37℃诱导 4 h 后, SDS-聚丙烯酰胺凝胶 (PAGE) 电泳后, 经扫描分析该融合蛋白产量占菌体蛋白总量 28.48%, 蛋白质印迹 (Western-blot) 证实了该融合蛋白的表达获得成功. 这为 BRD7 基因的蛋白纯化及抗体制备, 进一步开展其功能研究奠定了基础.

关键词 鼻咽肿瘤, 基因表达, BRD7 基因
学科分类号 R739.63

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