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Construction and Expression of Human Survivin and Preparation of Its Polyclonal Antibody

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Abstract Survivin, a novel member of inhibitor of apoptosis(IAP) protein family, is aberrantly expressed in cancer but undetectable in normal, differentiated adult tissues. The cancer-specific expression of survivin, coupled with its importance in inhibiting cell death and in regulating cell division makes it a useful diagnostic marker of cancer and a potential target for cancer treatment. Survivin cDNA amplified from the total RNA of 293 cells through RT-PCR was cloned into prokaryotic expression vector pRSET-B. The recombinant plasmid pRSET-B-Surv was expressed in *E.coli* BL21, and the relative molecule mass(M_r) of expressed fusion protein was approximately 21000. The recombinant protein was purified through Ni^{2+} affinity chromatography column and characterized by SDS-PAGE and Western blot. The purified recombinant protein was then injected into rabbits, and antisurvivin polyclonal antibody with a high titer was obtained.

Keywords Clone; Survivin; Expression; Polyclonal antibody

1 Introduction

Survivin[1], the smallest member of the inhibitor of apoptosis protein(IAP) family, is highly expressed in virtually all types of cancers, as well as in vascular endothelial cells during tumor associated angiogenesis but is undetectable in nonproliferating normal adult tissues[2]. The cancer of overexpression of survivin shows its stronger aggressive behavior, such as the decreased response to chemotherapeutic agents compared with cancers that are survivin negative[3], which suggests survivin has a potential role in tumorigenesis[4], and it has become a promising target for vaccination purposes[5,6].

The researches about survivin are under intense state in cancer gene therapy. However, why this protein is upregulated and what is the molecular mechanisms of regulation in cancer remain largely unknown[7].

Cloning and expression of human survivin and preparation of its polyclonal antibody are helpful to further researches for the molecular mechanism of survivin to inhibit apoptosis and understand the significance for clinical practice.

In this study, human survivin cDNA was cloned into the prokaryotic expression vector pRSET-B, and the recombinant expression plasmid, pRSET-B-survivin(pRSET-B-Surv), was constructed. Recombinant protein was expressed and purified, and survivin polyclonal antibody with high titer was obtained.

2 Experimental

2.1 Materials

293 cells, *E. coli* strains TOP10 and BL21, and pRSET-B vector were obtained from our laboratory. The pGEM-T easy vector was purchased from Promega. TRIzol reagent was purchased from Lifetechnologies. Restriction enzymes, T4 DNA ligase, and *Taq* DNA polymerase were purchased from New England Biolabs. Freund's complete adjuvant(FCA) and Freund's incomplete adjuvant(FIA) were purchased from Dingguo. The mAb against survivin was purchased from Novus-Biologicals. DL2000 marker and 1 kb DNA marker were purchased from Takara. Prestained protein marker was purchased from BioLabs and unstained protein molecular weight marker was purchased from Fermentas.

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2.2 Methods

2.2.1 RNA Isolation and Reverse Transcriptase-Polymerase Chain Reaction(RT-PCR)

Total RNA was isolated from 293 cells by the method of Horikoshi and Sakakibara[2]. Total RNA was extracted from 293 cells, and cDNA of survivin was synthesized by reverse transcription using Super-Script™ reverse transcriptase kit(Gibco). First-strand synthesis was performed using random hexanucleotides as primer, and PCR was performed with forward(5′-CTgCAgTCgACgCCgCCACCATgggTgCC-CCgACgTTg-3′) and reverse(5′-ggATCCggTACCA-AgCTTAATCCATggCAgCCAgC-3′) primers at annealing temperature adjusted to 55 °C. PCR products were subsequently examined by agarose gel electrophoresis, yielding a 465 bp product.

2.2.2 Plasmids Construction

The cDNA of survivin amplified from total RNA of 293 cells by RT-PCR were cloned into the pGEM-T vector. After confirming the size and sequence of survivin cDNA by restriction enzyme digestion and sequencing the recombinant plasmid pGEM-T-Surv, the cDNA of survivin was subsequently subcloned into *Pstl*/*Hind*III sites of pRSET-B vector.

2.2.3 Expression, Purification, and Identification of Survivin

The recombinant express plasmid pRSET-B-Surv was transformed into *E. coli* BL21. The recombinant protein with N-terminal with 6His-tagged survivin was induced by 1 mmol/L isopropyl-*β*-*D*-thiogalactopyranoside(IPTG) at an optical density of 0.4—0.6 at 37 °C for 5 h's expression. Bacterial lysates were prepared by sonication in buffer A(50 mmol/L Tris-HCl, pH 7.8, and 300 mmol/L NaCl, 8 mol/L urea). After centrifugation at 12000 r/min for 30 min, the supernatant was applied to a Ni^{2+}-nitrilotriacctate (Ni-NIA) agarose column(QIAGEN), washed with buffers B and C(their composition is the same with buffer A but with pH 6.0 and 5.3, respectively). Then, the recombinant protein was eluted with buffer D(its composition is the same with that of buffer A but with pH 4.0). The eluted solution was detected by SDS-PAGE, and Western blot with the mAb against survivin(Novus-Biologicals).

2.2.4 Western Blot Analysis

Survivin recombinant protein was degenerated by loading buffer(100 mmol/L DTT, 0.1% Bromocresol Blue, 2% SDS, 50 mmol/L Tris-HCl pH 6.8, 10% glycerol) and boiled for 5 min and then centrifuged for 10 min. The protein was loaded onto the gel and submitted to SDS-PAGE, and the resolved proteins were transferred to nitrocellulose membrane. After blocking with 3% nonfat milk in PBS for 40 min, the membrane was slit into equal width and incubated with various primary antibody for 90 min at room temperature. The blot was then probed with AP-conjugated secondary antibody for 40 min and stained by NBT and BCIP.

2.2.5 Preparation and Identification of Antisurvivin Polyclonal Antibody

Polyclonal antibody against human survivin was generated *via* a standard immunization protocol whereby rabbits were immunized with purified recombinant survivin protein. Briefly, the purified fusion protein was used to immunize rabbits with 300 μg once a week by subcutaneous multiple sites injection on backside. The serum was collected from the marginal of ear vein before prime immunization as negative control; the serum was collected once a week after the fifth immunization. The rabbits were immunized seven times in all, and antiserum was collected three times. One week after the last injection, the rabbits were sacrificed and the serum was collected. The specificity and titer of all the serum samples were analyzed by Western blot in different dilution.

3 Results

3.1 Amplification of Human Survivin cDNA Fragment

The human survivin cDNA fragment was amplified by RT-PCR. The RT-PCR products were analyzed by means of electrophoresis with 1% Agarose gel. The fragment is 465 bp, and the size of the PCR product as shown in Fig.1 is the same as that expected.

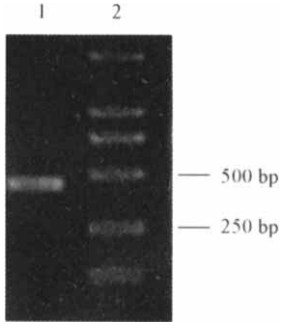

Fig.1 Agarose gel electrophoresis analysis of RT-PCR product of survivin cDNA

Lane 1: survivin cDNA produced by PCR; lane 2: DL2000 marker.