Production and evaluation of polyclonal rabbit anti-human p53 antibody using bacterially expressed glutathione S-transferase-p53 fusion protein

Mozhgan Rasti*, Zahed Khatooni, Zoherh Mostafavi-Pour

Recombinant Protein Laboratory, Department of Biochemistry, Medical School, Shiraz University of Medical Sciences, P.O. Box 7134-845794, Shiraz, I.R. Iran

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Abstract
p53 is a key tumor suppressor gene that is targeted for inactivation during human tumorigenesis. In this study, we produced and characterized polyclonal anti-human p53 antibody. The cDNA encoding the complete human p53 protein was cloned into pGEX-4T-1 and expressed in *Escherichia coli* as a fusion protein with *Schistosoma japonicum* glutathione S-transferase (GST). The rabbits were immunized with the purified p53 recombinant protein. The obtained antisera were purified to increase the specificity of recognition. The sensitivity and specificity of the produced antibody was analyzed by enzyme-linked immunosorbent, immunoblot, immunofluorescence and chromatin immunoprecipitation assays. Enzyme-linked immunosorbent assay showed that immunization with purified GST-p53 produced the high titer (1:10000) polyclonal antibodies with high specificity. Anti-p53 antibody allowed the sensitive detection of native p53 protein in immunoblotting, immunofluorescence and chromatin immunoprecipitation assays. Our results showed that anti-GST-p53 antibody provides a good means for studying the p53 expression pattern and its binding ability to other proteins in tumors.
Keywords**:** GST-p53; Tumor; Immunoassays; Anti-p53 antibody

Correspondence to:* **Mozhgan Rasti, Ph.D.
Tel/Fax: +98 711 2303029
E-mail: rasti31@yahoo.com

INTRODUCTION

p53 is a key tumor suppressor gene that is targeted for inactivation during human tumorigenesis. Point mutations or deletions in the *p53* gene are found in approximately 50% of all cancers (Wang and Sun, 2010). The point mutations cause a dramatic increase in the stability of the protein which can gain new functions and in many cases it seems to lose its suppressor activity of p53 (Goh *et al*., 2011; Goldstein *et al*., 2011). In normal cells, p53 plays a crucial role in the negative regulation of cell growth in response to DNA damage and other stress-activated signaling pathways (Olsson *et al*., 2007). Indeed, p53 has been reported to be involved in regulating G1 and G2 arrest as well as controlling differentiation and apoptosis pathways (Wu and Levine, 1994).

Antibodies against p53 have been produced for diagnostic and laboratory investigation purposes through immunizing animals with synthetic or recombinant p53 proteins (Cachot *et al*., 1998; Midgley *et al*., 1992). Many experiments have also been done with GST-fusion proteins for producing antibody against bacterial (Leung *et al*., 2002; Toye *et al*., 1990) and human proteins (Ayyildiz, 1999). In this study, we described the production of a whole p53- glutathione S- transferase (GST) fusion protein as a soluble product in single-step purification. The use of a GST-p53 fusion protein as an immunogen has not been reported so far for production of p53 polyclonal antibodies. The

purified recombinant protein was used to raise very high titer anti-p53 polyclonal antibodies whose clinical usefulness was evaluated in the related laboratory studies. The prepared antibody can be useful for the study of expression and binding ability of mutated p53 protein to other proteins in various cancers.

MATERIALS AND METHODS

Cloning of p53 into pGEX-4T1 vector: A human pcDNA-p53 was kindly provided by Dr. A. Turnell (University of Birmingham, UK). The cDNA of p53 (Gene Bank ™ accession number NM-000546) was excised from this cloning vector through digestion with *EcoRI* (Fermentas, UK) and *XhoI* (Fermentas, UK) enzymes and sub-cloned into the corresponding sites of a pGEX-4T-1 vector (Amersham Biosciences, UK) for bacterial expression (Fig. 1). The recombinant plasmid was verified by restriction enzyme digestion and direct sequencing of the sense and antisense strands by the use of an ABI prism 3100 genetic analyzer.

Expression and purification of GST-p53 fusion protein: Competent *E. coli* strain BL21 (DE3) was transformed with either parental (without inserted DNA) or recombinant pGEX-4T-1 vector. Briefly, 100 ng of plasmid DNA was added to 25µl competent cells for 30 minutes on ice and then heat shocked at 42ºC. The growth media was added to the transformation mixture and incubated at 37ºC for 2 h in a shaking incubator. GST and GST-p53 proteins were expressed and purified according to the guideline provided by the manufacturer (Amersham, Biosciences, UK). A single colony of successfully transformed *E. coli* was picked up and grown overnight in Luria Bertani (LB), supplemented with 100 µg/ml ampicillin at 37ºC with vigorous agitation. The culture mixture was then inoculated to 1 liter of LB medium (1:10 dilution) containing ampicillin and cultured at 37ºC for 3 h. Subsequent protein expression was induced at the appropriate density of bacteria (A600 = 0.6-0.8) by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) (Sigma, Canada) to the cell culture at a final concentration of 1 mM. The bacterial culture was put back in the shaker at 30ºC for 3 h. The bacterial cells were harvested by centrifugation at 3000 ×g for 10 min at 4ºC and re-suspended in 15ml of cold lysis buffer containing phosphate-buffered saline (PBS), 1 mM EDTA (pH 8) and 1% Triton X-100. Bacterial lysate was sonicated 3 times on ice for 45 seconds with 2 min 'on-ice' in

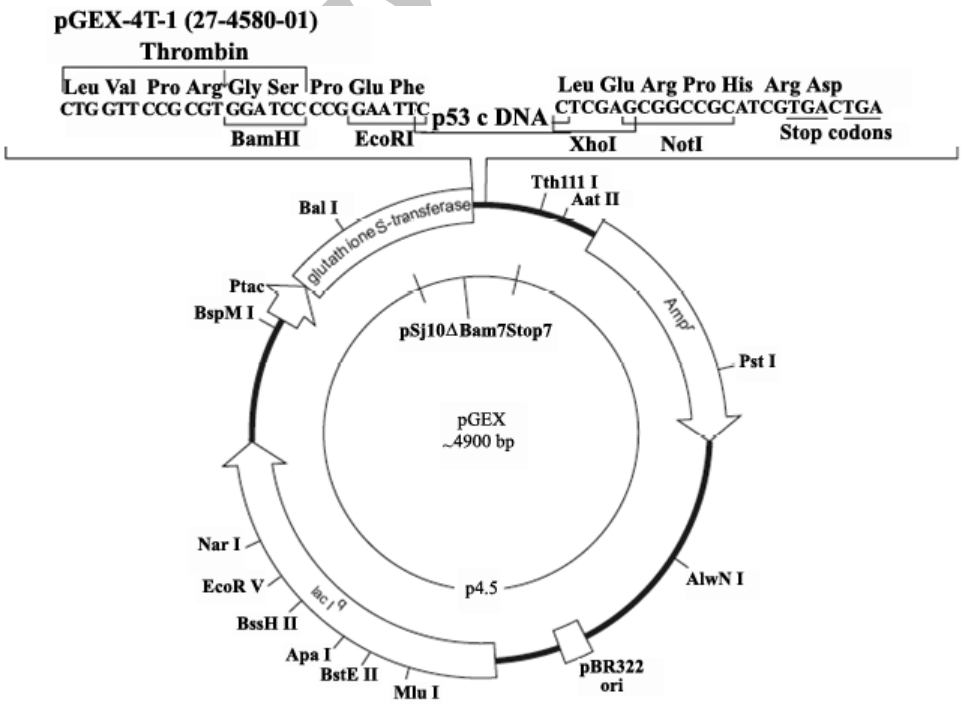

Figure 1. Map showing pGEX-4T-1-p53. The GST-p53 is expressed under the control of the inducible *tac* promoter for high-level expression and internal *laq1* gene for use in any *E.Coli* host.

between. The cell lysate was then centrifuged twice at 12,000 ×g for 15 min at 4°C to remove insoluble debris. The degree of expression was evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) before and after IPTG induction. According to the manufacturer's instructions, GST and GST-fusion proteins present in the supernatants were purified by affinity chromatography using glutathione-agarose (Sigma, USA). The GST and GST-fusion proteins were eluted from the glutathione-agarose by 25 mM reduced glutathione in 50 mM Tris-HCl solution pH 8.0. The purified proteins were dialyzed and their concentration was determined using a Bradford assay and quantified against a bovine serum albumin (BSA) standard curve (Bradford, 1976). The purity of protein products was analyzed with 12% SDS-PAGE and stored at -80°C.

Animal immunization: All the animal experiments were approved by the ethics committee of Shiraz University of Medical Sciences. Two male New Zealand white rabbits were simultaneously immunized by four intradermal injections on days 0, 14, 23 and 31. The first injection consisted of a mixture of 150 µg GST-p53 fusion protein in 0.5 ml saline with an equal volume of complete Freund's adjuvant. Subsequent injections consisted of 150, 75 and 50 µg GST-p53 proteins in 0.5 ml saline emulsified with an equal volume of incomplete Freund's adjuvant on days 14, 23 and 31, respectively. Before every immunization, blood samples were taken from rabbits and centrifuged. Then the sera were obtained. Generation of immune response was determined by enzyme-linked immunosorbent assay (ELISA). Five days after the last injection, antisera of the rabbits were harvested from the rabbit's carotid artery.

Evaluation of anti-GST-p53 antibody by ELISA: The titers of antisera were determined by an indirect ELISA. Each well of the 96-well microtiter plate was coated with 100 µl of 20 µg/ml GST-p53 fusion protein in carbonate buffer and incubated at 4°C overnight. After two washes with PBS, the wells were blocked using 200 µl of 1% BSA in 10 mM PBS for 2 hr at room temperature and then washed again twice with PBS. Polyclonal antibodies against GST-p53 protein were tested at dilutions of 1:2000, 1:5000, 1:7000 and 1:10000 in 1% bovine serum albumin. A 100 µl volume of each dilution of antisera was added to the well of a 96-well microtiter plate. The plate was covered with an adhesive plastic and incubated for 2 h at room temperature and then washed four times with PBS. A 100 µl/well of 1:2000 diluted horseradish peroxidase-conjugated goat antirabbit IgG (Sigma, Canada) was added and incubated for another 2 h in room temperature. After washing the plate, the wells were filled with 100 µl of a freshly prepared *O*-phenylenediamine dihydrocholoride (Sigma, Canada) substrate solution (1 mg/ml) and H_2O_2 and then incubated in the dark for 10 min. After adding stop solution to the wells, the absorbance was read at 492 nm with a microplate reader (Bio Tek, USA). The ratio of antiserum versus control serum absorbency was greater than 3 in each dilution.

Purification of polyclonal antibodies: Before performing immunoblot and chromatin immunoprecipitation analyses, the antisera of the rabbits were purified. This was done firstly by saturated ammonium sulfate (SAS) purification following standard protocol and then removing the anti-GST antibodies using GST immobilized on glutathione-Sepharose. Briefly, 100 µl of gluthione- Sepharose beads was coupled with 100 µg GST for 2 h on a rotator at 4°C. After coupling of GST, the beads were washed and centrifuged; 1 ml of 1/100-diluted SAS purified polyclonal antibody was added to the beads. It was incubated at 4°C overnight on a rotator. The purified polyclonal antibody was separated from beads by centrifugation at 3000 g and stored at -70°C.

SDS-PAGE and immunoblotting analyses: The samples containing the *E.coli*-produced GST fusion protein from different stages of protein purification and antisera were analyzed by 12% SDS-PAGE and stained with Coomassie Brilliant Blue. The purified anti-p53 antibody was tested for p53 recognition by immunoblotting. The MCF7 and MDA-MB-468 cells were used in this study. As MCF-7 cells express wild type p53 cells and MDA-MB-468 cells over-express p53 protein. Cells which had been solubilized in a lysis buffer containing 10 mM Tris-HCl, pH 7.4, 0.8 25M NaCl and 1% NP-40 were sonicated and cleared by centrifugation. Fifty-microgram protein samples including the prokaryotic expression product of GST-p53 and the cell lysate proteins were electrophoresed on 12% SDS-PAGE gel and electrophoretically transferred on to nitrocellulose membrane (Amersham Biosciences, UK) following the standard protocol. Immunoblotting was performed with the purified anti-p53 polyclonal antibody and commercial anti-p53 polyclonal antibody (ab17990; Abcam, Canada) as

golden standard. A horseradish peroxidase-conjugated anti-rabbit secondary antibody (Sigma, USA) and chemiluminescence substrates (ECL; Amersham Bioscience, UK) were used to determine the immunolabeled bands.

Cell culture: The estrogen receptor (ER) - negative MDA-MB-468 cells and ER-positive MCF-7 cells were used in this study. The human breast cancer cell lines, MDA-MB-468 and MCF-7, were grown in RPMI 1640 (Biosera, UK) supplemented with L-glutamine to 2 mM, and 10% V/V fetal calf serum (Cinagen, Iran) and sub-cultured 2-3 times a week.

Chromatin immunoprecipitation (ChIP) assay: The plates (10 cm) of MDA-MB-468 and MCF-7 cells were used in each assay. Formaldehyde was added directly to culture medium to a final concentration of 1% and incubated for 10 min at 37°C to cross-link histones to DNA. The ChIP assays were performed according to the manufacturer's instructions (Abcam, Canada). After lysing the cells, the mixtures were sonicated and sheered genomic DNA to 0.2-1 kb. ChIPs were carried out overnight at 4°C by mixing 5 µg of purified anti-p53, ab17990 and positive control anti-histone H3 (ab1791; Abcam, Canada) polyclonal antibodies. PCR reaction was used to amplify *estrogen receptor 1* (*ESR1*) promoter DNA that was bound to the immunoprecipitated histone. To visualize a specific PCR product, reactions were carried out in a total volume of 25 µl containing 2 µl of immunoprecipitated or total input, 0.5 µM of each primer (Cinnagen, Iran), 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate mixture (Fermentas, UK), 1x PCR buffer and 1.25 U of *Taq* DNA polymerase (Fermentas, UK). The *ESR1* promoter was analyzed using the forward 5'- TGA ACC GTC CGC AGC TCA AGA TC-3' and the reverse primer 5'-GTC TGA CCG TAG ACC TGC GCG TTG-3'. Amplification of this promoter was performed under the following conditions: 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 50 s; and a final extension of 10 min at 72°C. The amplified DNA was electrophoresed on 1.5% agarose gel and analyzed by ethidium bromide staining. All the ChIP assays were performed at least twice with similar results.

Immunofluorescence: For immunofluorescence microscopy of MDA-MB-468 cells, cells were grown on sterile glass coverslips and fixed in a cold (-15°C) mixture of methanol and acetone (1:1, v/v) for 7 minutes. The cells were washed three times with PBS and then blocked with 3% bovine serum albumin in PBS for overnight at 4°C. p53 was detected by incubating the cells with a dilution of 1:150 antiserum for 1 h. The cells were washed three times and then incubated with 1:200 dilution of goat anti rabbit IgG (Santa Cruz, USA) for 1 h. Coverslips were viewed on a Zeiss microscope (Axiostra plus, Germany), and immunofluorescence images were captured in the green (FITC) and blue (DAPI) channels using a Deltapix (Infinity X, Canada) camera. Advanced image analyses were performed using Adobe Photoshop CS4.

RESULTS

Cloning and expression of human *p53* gene: In order to express recombinant p53 in *E. coli*, complete human *p53* cDNA was subcloned from pcDNA3.1 vector into *EcoRI* and *XhoI* sites of a pGEX-4T-1 vector. This vector contains a coding sequence for glutathione-S-transferase (GST) in the N-terminal part. The integrity of the produced recombinant plasmid was confirmed by restriction mapping and sequencing. Aliquots of the sonicated bacterial cell lysates were analyzed by SDS-PAGE before and after IPTG induction (Fig. 2). As shown in Figure 2, after induction with IPTG, recombinant construct was expressed into *E. coli* BL21, producing a protein of approximately 80-kDa. The size of the produced protein was matched with the calculated

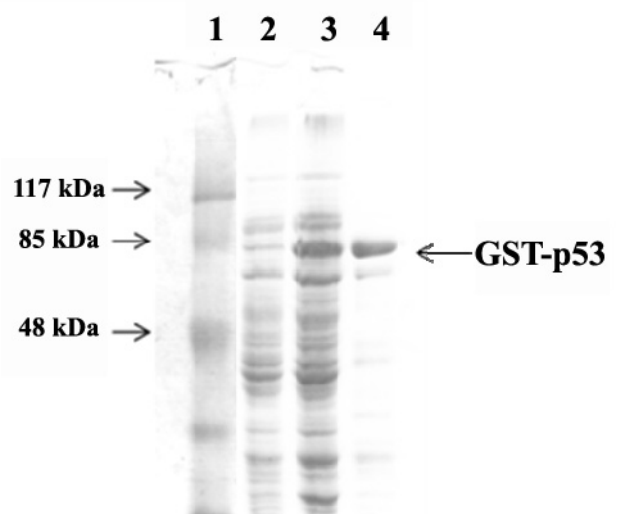

Figure 2. Coomassie Brilliant Blue stained SDS-PAGE demonstrating expression of recombinant GST-p53. Lane 1: molecular weight marker; Lane 2: lysate of uninduced BL21 cells containing pGEX-4T-1-p53 vector; Lane 3: lysate of the same cells with IPTG induction. Lane 4: supernatant after purification by affinity chromatography.

MW for GST-p53 protein; 26-kD is acquired from GST. Expression of the GST-p53 fusion protein was only observed in *E.coli* BL21 cells transformed with pGEX-4T-1-p53 but not from a vector without inserted DNA in it (Fig. 3). The GST-p53 fusion protein appeared as a single band on SDS-PAGE after purification by affinity chromatography using immobilized glutathione (Fig. 3). This new band reacted with commercial anti-p53 (ab17990) in immunoblotting assay (Fig. 4). The yield of recombinant GST-p53 was 1.6 mg per liter of bacterial culture.

Production of polyclonal antibodies: After immunization of the rabbits, high titers of anti-GST-p53 polyclonal antibodies were produced. It shows that the purified GST-p53 fusion protein was a potent immunogen. The titers of the obtained anti-GST-p53 sera were tested by indirect ELISA. Titers of the immune sera for 2 µg of the recombinant GST-p53 were estimated to be about 1:10000 (Fig. 5). The absorbance (A) ratio at 492 (A492 of post immunization sera/A492 of pre immunization sera) was greater than 3 in the highest dilution of the sera. Anti-GST-p53 polyclonal antibodies were purified by SAS precipitation and elimination of anti-GST by affinity purification using GST immobilized on glutathione-sepharose. Purified anti-p53 polyclonal antibody was evaluated by Commassia stained SDS-PAGE in comparison with antiserum (Fig. 6). Figure 6 shows evidence of anti-p53 antibody which was eluted on affinity purification. From the SDS-PAGE analysis of antiserum and anti-p53 polyclonal antibody, two main bands corresponding to the heavy (50 kDa) and light chains (25 kDa) of IgG are shown in Figure 6.

Evaluation of the anti-p53 polyclonal antibody by immunoblotting and immunofluorescence: For detection of antigenic specificity, the purified anti-p53 antibody was subjected to immunoblot analyses. Lysates of human breast carcinoma cell lines MCF-7 and MDA-MB-468 were used for immunoblotting with purified anti-p53 and commercial ab17990 antibodies (Fig. 7). A band of approximately 53 kD was observed in MDA-MB-468 cells by using both antibodies (Figs. 7A and 7B). However, p53 protein was only recognized by purified anti-p53 antibody in MCF-7 cells (Fig. 7B). MDA-MB-468 breast cancer cells contain a hemizygous mutant *p53* gene that overexpresses a stable p53 protein (Vinyals *et al*., 1999). However, MCF-7 human breast cancer cells have normal p53 function. Since MCF-7 cells express wild type p53, the low amount of p53 proteins in these cells is difficult to measure. For this reason, analysis of p53 bound by ab17990 gave a weaker result than MDA-MB-468 cells (Fig. 7A). However, our results for the produced anti-p53 polyclonal antibody demonstrated that this antibody could recognize endogenous p53 with high affinity in the two studied cell lines. As shown in Figure 4, the produced antibody could also recognize the p53 fusion protein with high sensitivity in comparison with commercial ab17990 in the same concentration of them.

In addition, immunofluorescence assay confirmed that the antiserum is able to stain the nuclei of the human breast carcinoma cell line MDA-MB-468 (Fig.

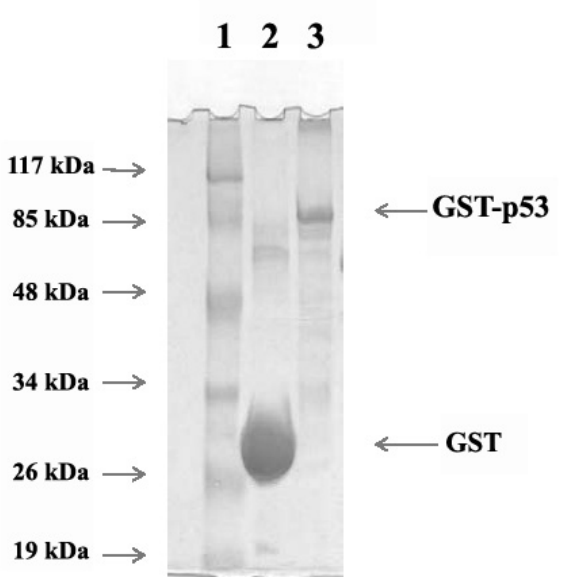

Figure 3. Bacterial expression of GST and GST-P53 proteins. 50 µg of purified GST and GST-P53 proteins were resolved by SDS-PAGE and stained with 0.1% (w/v) Coomassie brilliant blue. Lane 1: molecular weight marker; Lane 2: GST protein; Lane 3: GST-P53 protein.

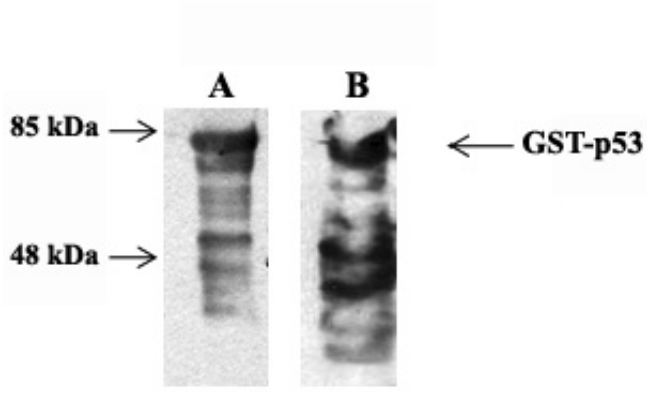

Figure 4. Immunoblot analysis of GST-p53 recombinant protein. 50 µg of fusion protein was subjected to SDS-PAGE and immunoblotted with A: anti –p53 (ab17990) and B: purified p53 polyclonal antibody. Arrow indicates 80 kD band for GST-p53.

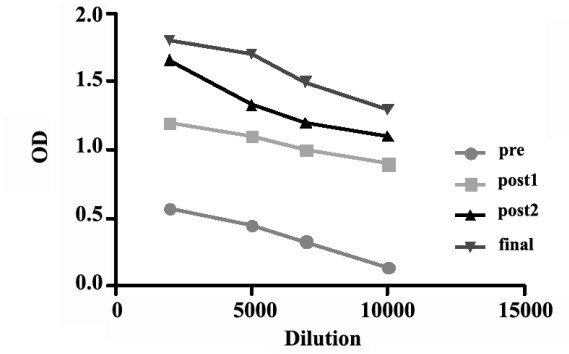

Figure 5. The titer of antiserum by ELISA. The immunoreactivity of the antiserum was tested at different dilutions of antibody (1:2000, 1:5000, 1:7000 and 1:10000) against 2 µg of the recombinant GST-p53. The antibody titer was found to be approximately 1:10000. Blood samples were taken from rabbits during four stages: pre; pre immunization, post 1; after first immunization, post 2; after second immunization, final; 5 days after final immunization. Each ELISA test was done two times.

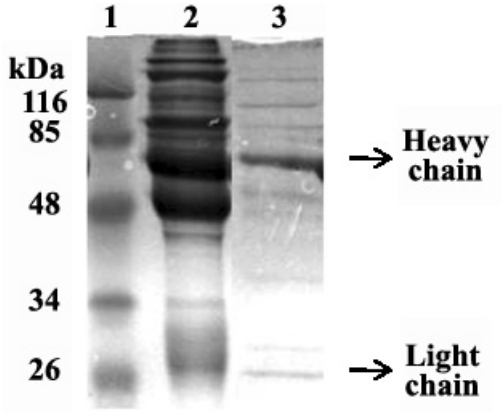

Figure 6. SDS-PAGE analysis of the purified anti-p53. Lane1: molecular weight marker; Lane 2: antiserum without purification; Lane 3: antiserum purified with SAS and affinity purification.

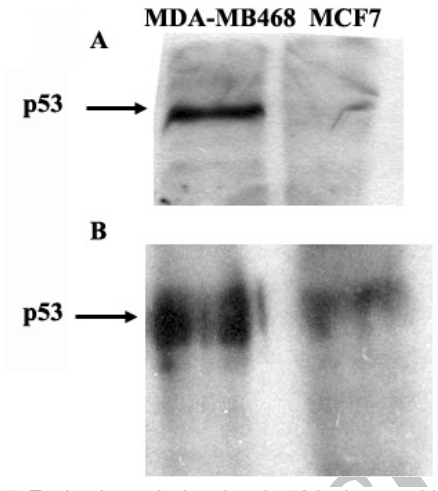

Figure 7. Evaluating polyclonal anti-p53 by immunoblot analysis. 50 µg of whole cell lysates from MCF7 and MDA-MB-468 cells were separated by SDS-PAGE and subjected to immunoblot analysis with A: ab17990 and B: purified anti-p53 polyclonal antibody.

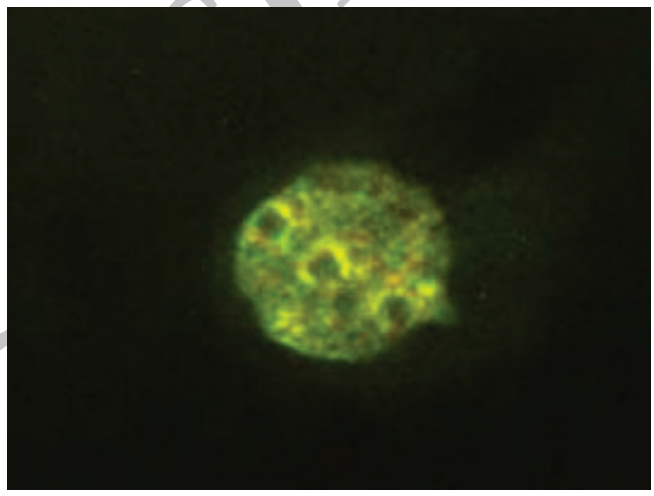

Figure 8. Immunofluorescence visualization of p53 with the anti-serum. Strong nuclear positivity of mutant p53 protein was observed in MDA-MB-468 human breast cell line.

8) which produces high amount of p53 proteins. Fig. 8 shows that the staining pattern was similar to what was seen with other anti-p53 antibodies on these cell lines. Like other anti-p53 antibodies there was an intense granular immunoflurorescence in the nuclei excluding nucleoli.

ChIP assays show p53 is associated with *ESR1* promoter CpG island: The ER-positive MCF-7 cells with an unmethylated *ESR1* promoter (Lapidus *et al*., 1998) were used in ChIP assays. The specificity of the produced polyclonal antibody was compared with ab17990 for binding to chromatin. Antibodies against p53 were used for immunoprecipitation of formaldehyde cross-linked protein-chromatin complexes from MCF-7 cells. In parallel, anti-histone H3 antibody which is enriched with chromatin was used as positive control. Immunoprecipitated DNA was analyzed for the presence of the *ESR1* gene by PCR using primers spanning a CpG island in its first exon region (Sharma *et al*., 2005). Figure 9 shows that p53 is associated with the active *ESR1* promoter in MCF-7 breast cancer cells. As shown in Fig. 9 both produced anti-p53 and ab17990 polyclonal antibodies exhibit the same results in ChIP assays. Histone H3 which was used as positive control was also observed at the *ESR1* promoter in MCF-7 cells.

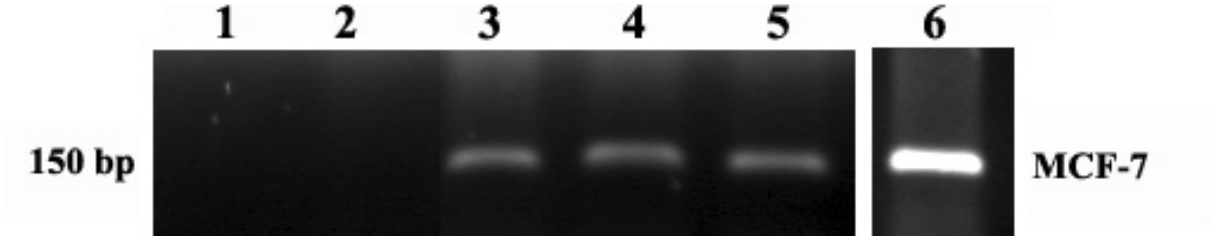

Figure 9. Recruitment of p53 at the Human ESR1 CpG island. Cross-linked chromatin prepared from ER-positive MCF-7 human breast cancer cells was immunoprecipitated with antibodies for histone H3 and p53. The immunoprecipitates were analyzed by PCR for ESR1 promoter CpG island. Lane 1: no antibody controls; Lane 2: negative controls reaction without DNA; Lane 3: anti histone H3 as positive control; Lane 4 and 5: association of purified p53 polyclonal antibody and ab17990 to ESR1 promoter, respectively; Lane 6: aliquots of chromatin taken before immunopercipitation were used as input controls.

DISCUSSION

In this study, we produced and characterized the polyclonal anti human p53 antibody in order to investigate the function of p53 protein. Whole human cDNA of p53 was used for making recombinant antigen into pGEX-4T-1 expression vector. This prokaryotic expression vector has the advantage of having a *tac* promoter upstream of the *Schistosoma japonicum* GST sequence. Thus, high level expression will only be induced when the lactose analog IPTG is added to the growing cell culture. In addition to the high-level expression, the vector allows mild elution conditions for release of fusion protein from the affinity medium. Therefore, the expression system we employed was very efficient as large amounts of a soluble cytoplasmic GST-p53 fusion protein were produced. The fusion protein contains GST moiety at the amino terminus and human p53 at the carboxyl terminus. GST moiety allows the purification of GST-tagged recombinant protein by affinity chromatography using immobilized glutathione. GST-p53 fusion protein was easily purified under non-denaturing conditions. 1.6 mg of GST-p53 fusion protein per liter of bacterial culture was obtained, and following elution with reduced glutathione, the fusion protein was essentially free of *E. coli* protein. As revealed by SDS-PAGE, the molecular weight of the recombinant GST-p53 was approximately 80-kDa (Fig.3). This value was greater than the native p53 size; the size difference was attributed to the presence of 26-kD GST protein.

The purified GST-p53 protein was used in the production of polyclonal antisera in rabbits. We made an attempt to evaluate the polyclonal anti-p53 by using ELISA and found that it specifically recognizes p53. Antiserum analysis by ELISA showed that we produced a high titer polyclonal antibody specific against the recombinant GST-p53 protein. The availability of large amounts of recombinant protein has allowed us to generate very high titer polyclonal antibodies to GST-p53. This experiment showed that the bacterial expressed protein increased specific recognition and antigenicity. Although bacterial expressed recombinant antigens do not have posttranslational modifications occurring in humans, this could not decrease the efficiency of this recombinant protein for immunogenicity in rabbits. Similar experiments have also been done with GST-fusion proteins for producing antibody against bacterial (Leung *et al*., 2002; Toye *et al*., 1990) and human proteins (Ayyildiz, 1999). However, different expression vectors have been used for making polyclonal antibody against p53 (Cachot *et al*., 1998; Midgley *et al*., 1992). It was previously reported that rabbit polyclonal anti-p53 antibody was raised against recombinant human p53 expressed in *E. coli* by a pT7-7 vector (Midgley *et al*., 1992). In this previous study, most of the recovered p53 protein was insoluble. In the present study, we were able to produce GST-p53 antigen as a soluble cytoplasmic protein. Soluble recombinant GST-p53 was easily purified and used as a potent immunogen for production of high titers anti-GST-p53 polyclonal antibody after an immunization protocol.

The antibody was elicited by rabbits immunization with GST-p53 which was capable of immunoprecipitating native p53. The antibody was used in immunoblotting, immunofluorescence and ChIP analyses. The sensitivity of the antibody against p53 was confirmed by the immunoblotting assay. Thus, this new antibody recognized the bands matching in molecular weight with the native and denatured p53 in two different cell lines with high sensitivity. In addition, by using adequate concentration of this antibody we could reduce the back ground of the immunoblots (Fig. 7). In addition, we determined the ability of the

produced antibody for specificity and sensitivity detection of p53 in immunofluorescence and ChIP assays. In this study, the antisera were bound to stable p53 in the nucleuses of MDA-MB-468 cells in immunofluorescence assay. Meanwhile, the binding of p53 to *ESR1* promoter in the ER-positive MCF-7 cells with an unmethylated *ESR1* promoter (Lapidus *et al*., 1998) was examined. Our ChIP experiments showed that p53 protein binds to the *ESR1* promoter CpG island in the ER-positive MCF-7 after treatment with anti-p53 antibodies (Fig. 9). We observed no difference between the results of using produced and commercial antibodies in this analysis. One of the genes regulated by p53 is the *ESR1* gene. It has been shown that p53 up-regulates *ESR1* gene expression by increasing its transcription (Angeloni *et al*., 2004). The binding of this protein to *ESR1* gene might contribute to its role in the regulation of transcriptional activity of this gene in MCF-7 cells. This finding is consistent with previous results for MCF-7 cell line (Akaogi *et al*., 2009; Shirley *et al*., 2009).

Polyclonal antibodies made by GST fusion proteins of bacteria have been used for immunological diagnosis of bacterial infections and in the construction of a bacterial vaccine (Leung *et al*., 2002; Toye *et al*., 1990). In addition, polyclonal anti-p53 antibody raised against recombinant human p53 was applied in many immunoassay experiments such as immunohistochemical staining of p53 in formalin–fixed tumor tissues (Midgley *et al*., 1992). Our experiments revealed that our produced antibody could recognize the p53 protein with high sensitivity in comparison with commercial ab17990 in the same concentration. However, it may have lower specificity than the commercial antibody. Such as the other laboratories that make their need antibodies, we produced about 50 ml antiserum using two rabbits which was enough for our current use in our lab. We anticipate that with the specific purification of antiserum by immunoafinity purification using protein a agarose, we are able to obtain a good quality antibody with more specificity for using it in diagnosis purposes.

In recent days, one key problem with obtaining and storing antibodies is their instability. Specially, this problem is getting worsened in hot climates. Another problem is their accessibility due to transportation problems, price inflation, customs delay among other factors. However, we showed that making polyclonal antibody by GST-fusion protein can solve all these problems and provide a good recourse for current studies with this antibody which has low cost and better sensitivity than the commercial one.

These results indicate that the use of recombinant GST-p53 antigen generated in prokaryotic expression system did not lead to variations in the antigen antibody recognition reaction for p53. The purified polyclonal antibody produced from the GST-p53 fusion protein is a specific, sensitive and useful alternative for the detection of p53 protein expression in tumor cell lines and resection samples.

In conclusion, we successfully produced polyclonal anti-human p53 antibodies by immunization of rabbits using GST-p53 fusion protein. The availability of the purified GST-p53 antigen allowed production of high titer antibody for diagnostic and clinical laboratory research purposes. We also found that the polyclonal antibody was effective in enzyme-linked immunosorbent, immunoblotting, immunoflurescence and chromatin immunoprecipitation assays for the quantification of human p53. We revealed that p53 protein presents on the promoter of *ESR1* in the ER-positive MCF-7 cells.

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Conflict of interest

None declared.

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