Preparation of monoclonal antibody to P53 and its clinical application*

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Abstract  Objective: The aim of this study was to prepare monoclonal antibody against P53, a kind of tumor suppressor protein, and use the antibody initially in clinical immunoassay. Methods: Monoclonal antibody was prepared and identified via the classic protocol of monoclonal antibody preparation. Identified monoclonal antibodies were purified by affinity chromatography. Antibody titer was determined by enzyme linked immunosorbent assay (ELISA). The specific binding activity of antibody was detected by Western blotting and immunohistochemistry. Results: Three strains of monoclonal antibodies named 1P15, 2P37 and 3P40 were obtained and purified by affinity chromatography. The purity of antibodies was higher than 90%. The titers of antibodies were more than 1: 6000. Western blot and immunohistochemistry assay showed that the specific antibody can combine with endogenous P53 protein in the tumor cell lines and determine the expression of P53 in tumor tissue. Conclusion: Three strains of monoclonal antibodies with high affinity to P53 were successfully established, which can be used for detecting the expression of P53 in tumor cells or tissue.

Key words  P53 protein; monoclonal antibody; tumor

Materials and methods

Animal and main reagents
BALB/c mice, provided by experimental animal center of Military Medical Science Academy, animal license No.: SCXK (Army) 2007-004; rProtein A-Sepharose TM 4 Fast Flow and Protein G-Sepharose TM 4 Fast Flow (Amersham Company, USA); horseradish peroxidase labeled Goat-anti-mouse IgG, N-four methyl benzidine (TMB) (Beijing Zhongshan Jinqiao Biotech Corporation, China); cellulose nitrate membrane (Millipore Company, USA); 96 cell culture plate and ELISA plate (Costar Company, USA).

Methods
Preparation and purification of monoclonal antibodies to P53
Using classical hybridoma techniques, 3 hybridoma cell strains stably secreting monoclonal antibody against P53 were obtained, named 1P15, 2P37 and 3P40. The...
cultured cells of the 3 cell strains were collected and injected into BALB/c mice intraperitoneally. Harvesting ascites, then 1P15 was purified with rProtein A-Sepharose TM 4 Fast Flow, 2P37 and 3P40 purified with Protein G-Sepharose TM 4 Fast Flow.

The titer of purified antibody determined by ELISA

The 96 well microtiter plate was coated with P53 protein antigen at a final concentration of 4 μg/mL, 100 μL/well, kept at 4 °C overnight. Discarding the excess antigen, the microtiter plate was blocked with 5% skim milk and incubated at 37 °C for 40 min. The purified antibody diluted at 1:400, 1:800, 1:1600, 1:3200, 1:6400, 1:12800 was added 100 μL per hole and incubated at 37 °C for 1 h. Washing away the excess antibody, 100 μL HRP labeled Goat-anti-mouse IgG was added and incubated at 37 °C for 1 h. Coloration by TMB, the absorbance value of 450 nm (A_{450 nm}) was read.

Determination of binding specificity of the antibody and P53 protein by Western blotting

The handled P53 protein samples was loaded onto 12% SDS-PAGE and blotted onto nitrocellulose membrane (Amersham) with transfer buffer (25 mM Tris, 190 mM glycine, 20% MeOH, 0.05% SDS, pH 8.3). The membrane was blocked with 5% skim milk in TBST (10 mM Tris, 100mM NaCl, 0.1% Tween 20, pH7.5) for 1 h and incubated with each McAb diluted 1:1000 (v/v) in TBST at 37 °C for 1 h. Then the membrane was washed, incubated with HRP conjugated goat anti-mouse IgG antibody diluted 1:1000 (v/v) in TBST at 37 °C for 45 min. After being washed with TBST for 5 min × 6, the membrane was detected by ECL.

Western blot determination of binding activity of 3P40 with intracellular P53 protein

The cultured MDA-MB-231 cells (breast cancer cell, belong to p53 mutant cell line) and H1299 cells (nonsmall lung cancer cell, belong to p53 deficiency cell line), were inoculated into 6 well plates with 1 × 10^4 cells per well, cultured at 37 °C, 5% CO_2 for 24 h. The cells were collected and the total protein was extracted. Thirty μg protein sample was detected in accordance with the above Western blotting procedure.

Immunohistochemical determination of binding activity of 3P40 with P53 protein in tumor tissues

Recruiting 126 cases of tumor specimens from 2009-01-10 to 2010-12-10 preserved in the clinical pathology department of our hospital, all data had complete record and clear diagnosis, including 19 cases of esophageal cancer, 25 cases of breast cancer, 23 cases of non-small cell lung cancer, 18 cases of gastric cancer, 16 cases of colon cancer, 12 cases of bladder cancer and 13 cases of prostate cancer. All specimens were sliced 4 μm thickness, stained by Envision method, observed by HE staining, PBS instead of the first antibody as negative control. P53 protein is a nuclear staining, positive staining is light yellow, brown or tan (Figs. 1, 2). At least 5 fields of each slice were chosen to calculate cells under the microscope, result assessment as follows: positive cells < 5%, negative; 5%–24%, positive (+); 25%–50% (++; > 50% (+++).

Results

Purification of the monoclonal antibodies to P53

Protein G-Sepharose TM 4 Fast Flow can highly purify 2P37 and 3P40, while rProtein A-Sepharose TM 4 Fast Flow could highly purify 1P15. The purity of the antibody reached above 90% with SDS-PAGE analysis.

Binding activity of monoclonal antibodies against P53

Fig. 3 showed that the 2P37, 3P40 and 1P15 were able to combine with the P53 protein. Three antibodies titers were measured by ELISA, among them 3P40 was the highest, up to 1:12000 above. Fig. 4 showed, 3 McAbs could be applied to detect P53 protein with Western blot.

Binding activity of P53 monoclonal antibody with endogenous P53 protein in tumor cells

Fig. 5 showed that 3P40 could specifically recognize P53 protein in MDA-MB-231 cell with a positive result. While H1299 cells belonging to the p53 defect type, so the result of Western blot was negative, illustrating the combination of 3P40 with endogenous P53 protein in the tumor cells at high specificity.

P53 monoclonal antibodies were used for immunohistochemical determination of P53 protein in tumor tissue specimens

Immunohistochemical assay 126 cases of tumor tissue specimens, including 19 cases of esophageal cancer, 25 cases of breast cancer, 23 cases of non-small cell lung cancer, 18 cases of gastric cancer, 16 cases of colon cancer, 12 cases of bladder cancer and 13 cases of prostate cancer, the positive rates were 63.2%, 64.0%, 60.9%, 33.3%, 68.8%, 83.3% and 69.2% respectively. Among the specimens, the positive rate of bladder cancer was the highest (83.3%), gastric cancer was the lowest (33.3%). Fig. 1 and Fig. 2 showed P53 positive results in the gastric cancer and lung cancer.

Discussion

P53 is so far found one of the highest correlated factors with the human tumors. P53 is involved in DNA damage repair, cell cycle regulation, apoptosis and inhibiting angiogenesis, known as the “gene guard” [9]. Mutations in the p53 gene will cause the loss of function, leading to the
formation of tumor. There are more than 60% of the tumor caused by p53 gene mutation. p53 mutation occurred in gastric cancer, liver cancer, colorectal cancer, bladder cancer, breast cancer, prostate cancer, lymphatic hematopoietic system tumor, glioma, soft tissue sarcoma, and malignant tumor [10–17] so on. Targeting P53 protein function reconstruction for clinical anticancer drug research has important significance.

Wild type human P53 protein expression in prokaryotic used as immune antigen, via classical hybridoma techniques, 3 hybridoma cell strains stably secretning monoclonal antibody against P53 were obtained in our study. ELISA and Western blotting identified that the 3 mAbs could specifically bind P53 proteins, among them the binding activity and detection sensitivity of 3P40 was the highest. 3P40 could specifically recognize the endogenous P53 protein in the tumor cells. The results of different immune staining showed that positive result mainly occurs in the nucleus, truthfully reflect the distribution of endogenous P53 protein, and consistent with the protein as a transcription factor function. The preliminary clinical application of 3P40 showed that it can combine specifically with a variety of P53 protein in tumor. Followed the further research of the function of P53 protein and P53 protein as anti-tumor drugs, specific monoclonal antibody against P53 protein will provide effective method for immune analysis and drug detection.

References


