Basic Investigations

MONOCLONAL ANTIBODIES AGAINST HUMAN GASTRIC CANCER

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Spleen cells from Balb/c mice immunized with five human gastric cancer cell lines in sequence were fused with murine myeloma cell line SP2/0, and hybridomas 5F4, 3GO and 3HI1, secreting monoclonal antibodies (MoAbs) against gastric cancer, were obtained through selective culture and screening. These MoAbs have both good selectivity and a high positive rate of reaction for gastric cancer, reaching 5/5 and 84.8% to 93.5% for gastric cancer cells and tissues respectively. The reaction of MoAbs with normal cells and tissues was negligible.

The corresponding antigens of the MoAbs were sensitive to digestion by trypsin and pronase and resistant to treatment with sodium periodate, indicating their nature as proteins. The antigen 3G9 could be visualized with Western blotting as two bands with molecular weights of 100KD and 70KD, however no band was found for antigens 3F4 and 3HI1. There was a high expression of antigens in the majority of gastric cancer cells and tissues independent of histopathological type of gastric cancer. A low expression of antigens was seen with other tumors and fetal gastrointestinal tissues. These could be considered as gastric cancer-associated antigens or oncofetal antigens with a quite extensive distribution.

Several types of monoclonal antibodies (MoAbs) against human gastric cancer have been produced and characterized at our institute.1-5 Although these MoAbs have good selectivity in distinguishing gastric cancer cells from normal cells, the positive reactivity rate with gastric cancer was not as high as expected. We have now developed three MoAbs through the multiple immunization of five human gastric cancer cell lines in sequence, and these have been proven to have both good selectivity and a high positive rate of reaction with gastric cancer cells and tissues. The corresponding antigens were also analyzed.

MATERIALS AND METHODS

Cells and Tissues

Human gastric cancer cell lines MGC803, SGC7901, M85, KatoIII, MKN45 and human fetal lung fibroblast cell line 2BS were cultured in DMEM, supplemented with 20% fetal calf serum (Gibco, USA). Human normal peripheral lymphocytes, red blood cells and bone marrow cells obtained from donors and a variety of normal and pathological tissues obtained from surgical specimens were identified by histopathological examination. Tissues were also obtained from 8-14 week old fetus' from induced abortions. All the tissue specimens were coated with OCT (Miles, USA), frozen in liquid nitrogen, and stored at -70° C.

Production of MoAbs

Six to eight weeks old, Balb/c mice, were immunized once a week with one of the human gastric cancer cell lines: M85, SGC7901, KatoIII
or MGC803, in sequence (5 × 10⁶, ip). At intervals of four weeks, the mice were boosted intrasplenically (2 × 10⁵) with the other gastric cancer cell line, MKN45. After three days, the spleen cells were removed and fused with murine myeloma cells sp2/0. The grown hybridomas were screened by using indirect immunoperoxidase or immunofluorescence stain. The clones whose supernatants had positive reactions with the five gastric cancer cell lines but negative with normal lymphocytes, red blood cells, bone marrow cells and fibroblasts were selected. After subcloning three times, hybridomas 3F4, 3G9 and 3H11 were obtained which secreted MoAbs against target cells constantly. These hybridomas were injected intraperitoneally into pristane primed Balb/c mice, and ascitic fluid rich in MoAbs was collected 7-10 days later. The subclasses of MoAbs 3F4, 3G9 and 3H11 were identified by double immunodiffusion as IgG1, IgG1 and IgG2b respectively.

ABC Immunoperoxidase Stain

Frozen tissue sections of 4-5 μ were fixed with cold acetone for 2-3 minutes. After washing with PBS, tissue sections were incubated with hybridoma supernatants or ascitic fluid at a 1:200-1:500 dilution in a humid chamber for one hour. The tissue sections were then stained with ABC Kit (Vector, USA). Two researchers separately reviewed the slides and characterized the stain as a percentage of cancer cells stained (range), intensity of stain and location. Stain range was divided into 10-25%, 25-50%, 50-75% and 75-100%. Intensity was evaluated as negative (−), yellow color positive (+) and brown color positive (++). The stain could be located in the membrane, cytoplasm or both.

Immunoblotting Assay for Antigens

M85 cells (10⁷) were frozen and thawed twice until 95% of the cell membranes were broken but the nuclei were intact. The remaining intact cells and nuclei were eliminated by centrifugation at 600 g for 20 minutes. Subsequent centrifugation at 100,000 g for one hour (4°C) produced a crude cell membrane pellet. Lysate buffer 200 μl (0.5% NP-40, 50 mM NaCl, 10 mM PMSF, 1 mM EDTA in 20 mM Tris-Hcl buffer, pH 8.2) was added to the pellet, and the mixture was shaken for 15 minutes at 4°C. Following repeat centrifugation at 100,000 g for one hour, the supernatant was collected as an antigen extract.

Ten microliters of the antigen extract was spotted on to the nitrocellulose membrane and dried at room temperature. The spots were treated with trypsin (5 mg/ml) and pronase (0.6 mg/ml) for one hour at 37°C, and sodium periodate (1 mg/ml) for one hour at 4°C. After washing, the spots were blocked with 5% BSA-PBS. The MoAb supernatants, rabbit anti-mouse IgG conjugated with horseradish peroxidase (HRP-RdMuIgG) and a substrate solution containing DAB and H₂O₂ were added sequentially, with thorough washings between additions.

A parallel experiment omitting the enzyme or sodium peroridate treatment served as a control.

The antigen extract was run on a SDS-PAGE® (12.5% gel concentration, reduced and nonreduced conditions). After electrophoresis, the gel was immersed into a buffer solution (20% methanol, 20 mM Tris and 150 mM glycine, pH 8.0) for 10 minutes. The antigens in the gel were electroblotted onto a nitrocellulose membrane® (6-8 V/cm, 16-18 hours, 4°C). The blotted membrane was blocked with 5% BSA (containing 150 mM NaCl, 10 mM Tris-Hcl, pH 8.0) for 15 minutes then incubated with MoAb supernatant or ascitic fluid at a 1:500 dilution for 60 minutes at room temperature. The antigen bands were visualized by adding HRP-RdMuIgG (1:1000) and substrate solution as described above.