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A Novel Putative Tumor Associated Antigen*

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Abstract 17-1A is a tumor associated antigen. Monoclonal antibody (mAb) against 17-1A has been used in adjuvant therapy of colorectal carcinoma. Using mAb against 17-1A antigen on affinity chromatography, a novel putative tumor associated antigen (P50) whose relative molecular mass is 5.0×10^4 has been isolated from human colorectal tumor tissues which are recognized by mAbs 17-1A and M79, while the relative molecular mass of 17-1A antigen isolated from several colorectal tumor cell lines is 3.3×10^4. P50 was recognized by mAbs 17-1A and M79 which are specific mAbs against 17-1A antigen.

Key words 17-1A antigen; colorectal tumor tissue; tumor associated antigen

Introduction

Tumor associated antigen 17-1A is broadly distributed in normal epithelial tissues and is also found in various types of carcinoma[1]. 17-1A antigen isolated from several human colorectal carcinoma cell lines is a glycoprotein containing N-linked glycans with a relative molecular mass of 3.3×10^4[1,2], and is located on cell surface[3]. Monoclonal antibody 17-1A could mediate ADCC against colorectal carcinoma cells *in vitro*[3,4]. The mAb has been successfully used in adjuvant therapy on human colorectal carcinoma[5]. We have isolated a novel tumor associated antigen (P50) from human colorectal tumor tissues.

1 Materials and Methods

1.1 Human colorectal tumor tissues

4 samples of fresh human colorectal tumor tissues were obtained from China-Japan Friendship Hospital. Tissues were homogenized in lysis buffer (volume fraction of tissue in lysis buffer is 5%) for 3 min and lysed for 1 h at 4℃. The lysis buffer comprised 50 mmol/L Tris, 0.5% NP-40, 0.18 mg/L PMSF, 1.57 g/L Benzamidin-HCl, 0.1 g/L Trypsin inhibitor and 0.1% Leupeptin. Tissue lysates were prepared by centrifugation at 800*g* for 30 min and at 10 000*g* for 45 min.

1.2 Antibodies

mAb 17-1A and mAb M79 which are specific mAbs to 17-1A antigen(described in Ref.[1]) were kindly provided by Prof. G. Riethmuller (Munich, Germany). Fluorescein isothioacyanate conjugated and peroxidase-conjugated rabbit anti-mouse immunoglobulins were obtained from Dako.

1.3 Affinity chromatography

Using standard method from Pharmacia, mAb M79 (4 mg) was coupled to 2 mL CNBr-activated sepharose 4B. Using low flow rate, tissue lysates were run twice through a column filled with 2 mL M79-sepharose. The column was washed with 40 mL PBS, eluted with 2 mL 0.5 mol/L acetic acid at 4℃. Eluates were neutralized with 0.5 mol/L NaOH.

1.4 SDS-PAGE and silver-staining

Eluates prepared from tissue lysates absorbed using M79-sepharose were subjected to electrophoresis in a 12.5% SDS-polyacrylamide gel under reducing

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condition and then examined by silver-staining.

1.5 Enzyme-linked immunosorbent assay (ELISA)

The isolated protein P50 (1 mg/L) was coated overnight on a microtiter plate at 4℃. Nonspecific binding was blocked by incubation with 1% human serum albumin or 0.3% gelatin in PBS. After washing twice with PBS-Tween 20 (0.1% Tween 20), mouse mAb M79 or 17-1A was added and incubated for 1 h at room temperature. The plate was washed again with PBS-Tween 20. Peroxidase-conjugated rabbit anti-mouse antibody was added. After further washing, freshly prepared *o*-phenylenediamine-peroxide solution was added and the optical density (D) was measured (λ= 450 nm).

2 Results and Discussion

During the study of 17-1A antigen, a novel putative tumor associated antigen was separated from human colorectal tumor tissue lysates with M79 (an mAb against 17-1A antigen) affinity chromatography column. Using SDS-PAGE under reducing condition and silver-staining, we identified the protein with a relative molecular mass of 5.0×10^4 (Fig. 1), and named the protein as P50. In ELISA assay, two monoclonal antibodies against 17-1A tumor associated antigen, 17-1A and M79, could both recognize the protein P50 (Fig. 2). The relative molecular mass of the protein (P50) isolated from colorectal carcinoma tissues is clearly different from that of the antigen (P33, relative molecular mass of 3.3×10^4)[1,2] isolated by 17-1A and M79 affinity chromatography from human colorectal carcinoma cell lysates. P50 is probably a novel putative 17-1A tumor associated antigen. Reports on 17-1A antigen are not congruent with each other. Ross et al reported that 17-1A antigen contained a 30×10^3 subunit and a 40×10^3 subunit[6]. There is still another report that 17-1A antigen is a 41×10^3 protein[6]. P50 was isolated from colorectal tumor tissues, while other antigens were isolated from colorectal tumor cell lines. Understanding of the relationship among these antigens should be beneficial to the ajuvant therapy of mAb 17-1A on human colorectal carcinoma.

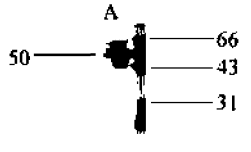

Fig. 1 Isolation of a protein (p50) from lysates of human colorectal carcinoma tissue by affinity chromatography
The column was filled with M79-sepharose. Lane A, eluate from tissue lysate; Lane B, protein weight marker.

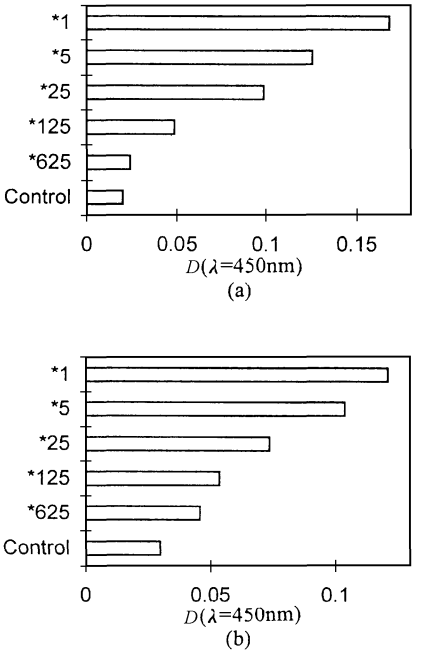

Fig. 2 Identification of the protein p50 in eluates by ELISA
(a) binding of mAb 17-1A to p50; (b) binding of mAb M79 to p50

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