RICINUS COMMUNIS AGGLUTININ II-REACTIVE GLYCOPROTEINS FROM THE ASCITES OF PATIENTS WITH HEPATOCellular CARCINOMA AND THEIR USE IN ENZYME-LINKED IMMUNOSORBENT ASSAY

Masaki FUKUSHIMA, M.D., Terukatsu ARIMA, M.D. and Hideo NAGASHIMA, M.D.
First Department of Internal Medicine, Okayama University Medical School,
Okayama 700, Japan

Summary

Ricinus communis agglutinin II-reactive glycoproteins from the ascites of patients with hepatocellular carcinoma were prepared using lectin affinity chromatography. Normal serum- and cirrhotic ascites-components were removed by columns with immobilized antibodies against them. Ricinus communis agglutinin II-reactive glycoproteins thus obtained were supposed to be hepatocellular carcinoma-associated and less than 0.1% of the protein in the starting material.

Polyacrylamide gel electrophoresis of these glycoproteins revealed more than 10 major polypeptides with molecular weights ranging from 20K to 200K daltons.

The rabbit antiserum raised against them reacted with at least three components of 45, 52 and 55K daltons. The serum level of this antibody-reactive glycoproteins was assessed by an enzyme-linked immunosorbent assay. It was elevated in 91% of cases of hepatocellular carcinoma, 70% of cases of other gastrointestinal carcinoma, 88% of cases of liver cirrhosis, 55% of cases of chronic hepatitis, and 25% of cases of acute hepatitis. The mean value of hepatocellular carcinoma was significantly greater than those of other groups. These results suggest that some of Ricinus communis agglutinin II-reactive glycoproteins in hepatocellular carcinoma patients may be cancer-associated glycoproteins and that their serum levels are increased in hepatocellular carcinoma patients.

Key Words: Cancer-associated glycoproteins, Enzyme-linked immunosorbent assay, Hepatocellular carcinoma, Lectin affinity chromatography, Ricinus communis.

Introduction

Cellular transformation is associated with changes of the carbohydrate chains of cellular glycoproteins and their synthetic rate. These glycoproteins may be secreted in the circulation, such as α-fetoprotein (AFP), or shed from the tumor cell surface as membrane fragments1. Increased serum levels of some glycoproteins are often observed in cancer patients2,3.

In the study of rat fibrosarcoma variants, the cell surface glycoproteins of highly tumorigenic cells had a larger quantity of galactose (Gal) or N-acetyl-galactosamine (GalNAc) residues at their non-reducing ends of carbohy-
drate chains than those of less tumorigenic cells\(^6\).

Ricinus communis agglutinin II (RCA II) is a lectin having an ability to bind terminal Gal and/or GalNAc residues of glycoproteins\(^5\). Using this lectin, we showed that serum level of glycoproteins having terminal Gal and/or GalNAc residues on their carbohydrate chains was elevated in patients with gastrointestinal carcinomas including hepatocellular carcinoma (HCC)\(^6\).

In the present study, we prepared RCA II-reactive glycoproteins from HCC ascites by RCA II affinity chromatography, and partially purified HCC-associated glycoproteins from them by removing normal serum- and non-malignant ascites-components. The antibody against them was raised. Serum levels of this antibody-reactive glycoproteins in liver diseases and carcinomas were determined by enzyme-linked immunosorbent assay (ELISA).

**Materials and Methods**

Ascitic fluid was obtained from four patients with HCC and one cirrhotic patient. Cells were removed by centrifugation. 163 serum samples were obtained from 26 normal cases, 42 HCC, 32 liver cirrhosis, 20 chronic hepatitis, 20 acute hepatitis, and 23 other gastrointestinal carcinoma cases. Ascitic fluid and serum samples were stored at \(-20^\circ C\).

**Preparation of Antisera**

Antisera to cirrhotic ascites (90 mg protein/ml) and to RBG-1 (see below, 1 mg protein/ml) were raised in rabbits. Protein solution was emulsified with an equal volume of Freund’s complete adjuvant and 1 ml of the emulsion was injected subcutaneously. Booster immunizations were performed on days 28, 35 and 42. They were bled 7 days after the last injection. Immunoglobulin was obtained by the ammonium sulfate precipitation method.

**Preparation of Immobilized Proteins**

The proteins in carbonate buffer (0.1 M NaHCO\(_3\), 0.5 M NaCl, ph 8.3) were covalently linked to CNBr-activated Sepharose 4B according to the manufacturer's instructions (Pharmacia Fine Chemicals, Uppsala, Sweden).

**Preparation of PBG-1**

Ascitic fluid from HCC patients was centrifuged at 22,000\(\times\)g for 30 min at 4\(^\circ\)C, and each 30 ml of the supernatant were applied to a column (2.6 X 11 cm) of RCA II-agarose (3-5 mg RCA II/ml gel, E. Y Lab., San Mateo, U.S.A.) equilibrated with 0.01 M phosphate buffer, pH 7.3, containing 0.15 M NaCl (PBS). The column was then washed with PBS at a flow rate of 10 ml/hr until the effluent showed an absorbance of less than 0.01 at 280 nm. The bound glycoproteins were eluted with 0.1 M \(\beta\)-lactose in PBS, and dialyzed against PBS at 4\(^\circ\)C.

The bound glycoproteins were then passed successively through two affinity columns, i.e., a rabbit anti-whole human serum antibody (DAKOPATTS a/s, Glostrup, Denmark)-conjugated Sepharose 4B column (2.6 X 13 cm, 15 mg IgG/ml gel) and a rabbit anti-cirrhotic ascites antibody-conjugated Sepharose 4B column (2.6 X 15 cm, 10 mg IgG/ml gel). The unbound glycoproteins finally obtained were designated RBG-1.

**Polyacrylamide Gel Electrophoresis**

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) was performed according to the procedure of Laemmli\(^7\) with 7.5% separating gel. Proteins were stained by the silver staining method of Oakley et al.\(^8\).

**Immunoblot Analysis**

Proteins were electrophoretically transferred from SDS-polyacrylamide gel to nitrocellulose membrane as described by Burnette\(^9\).

After transfer, the nitrocellulose membrane