Characterization of Tumor Specific Antigens on the Plasma Membrane Surface of Rat Hepatomas Induced by 3'-Me DAB and Identification of the Common Tumor Specific Antigens from Rat Hepatomas Induced by Different Chemical Hepatocarcinogens

Yoon Soo Kim, Kyung-Soo Hahm, Kyung Sup Kim and Nam Jeen Lee

Three different chemical carcinogens, 2-acetylaminofluorene (AAF), diethylnitrosamine (DENA), and 3'-methyl-4-dimethylaminoazobenzene (3'-Me DAB) were used to induce hepatomas in rats. Plasma membrane surface proteins of normal rat liver cells and rat hepatomas were extracted with 3M KCl. From the analysis of the proteins of normal rat liver and rat hepatoma induced by 3'-Me DAB by discontinuous polyacrylamide gel electrophoresis (Disc-PAGE), under nonreducing and nondenaturing conditions, polyacrylamide gel electrophoresis in the presence of SDS and 2-mercaptoethanol (SDS-PAGE), Sephadex G-200 gel permeation chromatography, DEAE-A50 ion-exchange chromatography and two-dimensional gel electrophoresis, at least three tumor specific antigens were identified. One had a molecular weight of 66,000 (pI=6.79) while the other two had the same molecular weight, 73,000 but differed in their isoelectric points (7.58 and 7.81). For immunological analysis of tumor specific antigens, the absorbed antiserum was prepared. Plasma membrane surface proteins of rat hepatoma induced by 3'-Me DAB were used to obtain New Zealand White male rabbit antiserum. Rabbit antiserum was then reacted with the proteins isolated from the plasma membrane surface of normal rat liver and the absorbed antiserum reacting specifically with the tumor specific antigens derived by 3'-Me DAB was obtained. Using the absorbed antiserum, the immunoreactivities of plasma membrane surface proteins isolated from rat hepatomas induced by 3'-Me DAB, AAF, and DENA were compared by Ouchterlony double immunodiffusion analysis and immunoelectrophoresis. To characterize the proteins reacting to the absorbed antiserum, immunoglobulin G was separated from the absorbed antiserum and coupled to cyanogen bromide-activated Sepharose CL-4B. The isolated proteins from the plasma membrane surface proteins of 3'-Me DAB-induced hepatoma using this immunofinity chromatography had molecular weights of 66,000 and 73,000. The localization of these proteins on surface plasma membranes of rat hepatomas induced by 3'-Me DAB was confirmed by an immunofluorescence technique. The experimental results revealed the existence of cross-reacting common antigens on the plasma membrane surface of rat hepatomas induced by different hepatocarcinogens.

Key Words: Tumor specific antigen, hepatoma, hepatocarcinogen affinity chromatography

During malignant transformation, changes are known to occur in the proteins of the plasma membrane surface and cause the transformed cells to possess different physiological properties when compared to normal cells. Since Paul Ehrlich (1908) first reported that tumor cells could be recognized as foreign by the host cell because of this unusual property, many studies have been devoted to tumor specific antigens which are the new proteins appearing on the cell surface during malignant transformation. It is also known that different types of cells and carcinogens cause different changes in plasma membrane surface proteins. The plasma membrane of an animal infected with an RNA tumor virus displayed a new protein called a gross cell surface antigen (GCSA) and this antigen was immunogenic to the host.
animal (Kurth et al. 1979).

There have also been studies on the tumor specific antigens induced by chemical carcinogens. Baldwin and Graves (1972) reported on a tumor specific antigen from the plasma membrane of an aminoazo dye (4-dimethylaminoazobenzene; DAB)-induced rat hepatoma. They used limited papain digestion to solubilize plasma membrane fractions isolated from the cells of a DAB-induced rat hepatoma and isolated a tumor specific antigen capable of neutralizing antibodies in a tumor-immune sera by DEAE cellulose chromatography. Natori et al. (1977) used Nonidet P-40 to isolate a fibrosarcoma specific antigen from mice fibrosarcoma induced with methylcholanthrene and reported that the molecular weight was 70,000.

On the other hand, Pellis et al. (1976) obtained a tumor specific transplantation antigen (TSTA) from 3M KCl extracts of 3-methylcholanthrene-induced murine fibrosarcoma after treating the tumor cells with trypsin and purified by ammonium sulfate fractionation and polyacrylamide gel electrophoresis (PAGE). They isolated two components showing TSTA activity which had isoelectric points of 5.05 and 6.90, and estimated molecular weights of 40,000 and 75,000, respectively.

Since the tumor specific antigens described above were obtained by using either a proteolytic enzyme or nonpolar detergent, these antigens may be a part of a molecule instead of being the complete one. Recently, a method using 3M KCl for isolating the complete form of an antigen from the plasma membrane surface was reported by Stevens et al. (1981), and by using this method, three tumor specific antigens were identified (Lim et al. 1982) on the surface of the plasma membrane of rat hepatoma induced by 3'-methyl-4-dimethyl-aminoazobenzene (3'-Me DAB).

The studies described herein were initiated to purify the tumor specific antigens identified from the plasma membrane surface of a rat hepatoma induced by 3'-Me DAB, to characterize their molecular properties, and to see whether common tumor specific antigens are formed when hepatomas were induced by other known hepatocarcinogens such as 2-acetylaminofluorene (AAF) and diethylnitrosamine (DENA).

**MATERIALS AND METHODS**

**Induction of Tumors:** Liver tumors were induced in male Spargue Dawley rats (about 80g) by the protocols shown in Table 1 using 3'-Me DAB (Eastman Kodak Co.), AAF (Sigma Chemical Co.), and DENA (Sigma Chemical Co.). The first group was given 0.02%

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>Method of Administration</th>
<th>Duration for Tumor Induction</th>
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<tbody>
<tr>
<td>2-Acetylamino-</td>
<td>0.02% AAF in a diet for 8 wks followed by 0.05%</td>
<td>48 wks</td>
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<tr>
<td>Fluorene (AAF)</td>
<td>phenobarbital containing diet.</td>
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<tr>
<td>Diethylnitrosamine</td>
<td>0.007% in the drinking water</td>
<td>30 wks</td>
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<tr>
<td>(DENA)</td>
<td>10 ml/day/cage of 5 rats for 5 days a week.</td>
<td></td>
</tr>
<tr>
<td>3'-Methyl-4-di-</td>
<td>0.06% in a diet for 12 wks followed by control</td>
<td>16 wks</td>
</tr>
<tr>
<td>Methylamino-</td>
<td>holosarcoma induced with methylcholanthrene</td>
<td></td>
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<tr>
<td>azobenzene (3'-Me DAB)</td>
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AAF (Peraino et al. 1971) in a synthetic diet (Lim et al. 1982) ad libitum for 8 weeks followed by a 0.05% phenobarbital containing diet, and a liver tumor was observed after 48 weeks of feeding. The second group was given DENA (Lijinsky and Taylor, 1978) in the drinking water (0.007%) for 30 weeks (100ml/day/cage of 5 rats for 5 days a week) ad libitum. Liver tumors were induced in the third group by feeding with 0.06% 3'-Me DAB in a diet for 12 weeks followed by a control diet ad libitum for 4 weeks (Lim et al. 1982). For each treatment group, an appropriate control group was maintained to obtain normal livers.

**Extraction of Plasma Membrane Surface Proteins:**

Extraction of surface proteins from the plasma membrane of liver cells of normal rats and bearing liver tumors was carried out using 3M KCl essentially according to the procedure of Stevens et al. (1981). The liver was removed from the rat under ether anesthesia after perfusing it with physiological saline containing 0.5mM CaCl₂.

Minced liver was washed by centrifugation at 5,000 x g for 30 min three times with phosphate buffered saline (PBS), pH 7.4 before extracting surface proteins with 3M KCl in PBS, pH 7.4 (2ml per g of tissue) for 18 hours at 4°C with constant stirring. The mixture was centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge; Du Pont Instruments) at 20,000xg for 30 min followed by centrifugation at 110,000 x g for 90 min (L-2-65B Ultracentrifuge; Beckman). The supernatant was dialyzed against PBS, pH 7.4 followed by 1mM EDTA-water, pH 7.4. The dialysate was centrifuged at 110,000 x g for 90 min and the clear supernatant was sterilized by passing through a membrane filter (0.45 μm; Gelman). The
solution was divided into 1.0 ml aliquots and stored frozen at -70°C. Protein concentrations were determined by the modified micromethod of Lowry et al. (1951) using bovine serum albumin (BSA) as a standard.

**Preparation of Immune Sera:** New Zealand White male rabbits (2kg) were used to obtain immune sera. The rabbits were immunized with plasma membrane surface proteins isolated from the liver tumor cells induced by 3'-Me DAB (500 μg protein per 2 ml of complete Freund adjuvant) followed by a booster injection of 300 μg protein in 1 ml of incomplete Freund adjuvant two weeks after the first injection. Serum was separated two weeks after the booster shot. Serum from normal rabbits was also separated after an 18 hr fast and used as the control serum. The rabbit antisera was reacted with control proteins isolated from the plasma membrane of normal rat liver (50 mg proteins per ml of antisera) at 37°C for 1 hr and the absorbed serum specific for tumor associated antigen was separated by centrifuging at 8000 x g for 10 min. The clear supernatant was passed through 0.45 μm membrane filter and stored at -70°C in 1 ml aliquots.

**Immunofluorescence Analysis:** Localization of tumor specific antigens on the plasma membrane surface of rat hepatoma induced by 3'-Me DAB was confirmed by the immunofluorescent technique. Mono-cellular suspensions of normal rat liver and rat hepatic tumor induced by 3'-Me DAB were prepared by the enzymatic method using hyaluronidase according to the procedure described by Howard et al. (1967). The cell suspension in calcium free Hanks' solution were smeared on the slides and the absorbed rabbit antisera (1:10 diluted) specific for 3'-Me DAB-induced hepatoma was added. After incubating the slides at 40°C for 30 min, goat anti-rabbit IgG-FITC (1:80 diluted) was added and the slides were incubated at 40°C for 30 min. The slides were then stained with Evans blue, were viewed under a UV lamp and picture were taken.

**Electrophoresis:** Discontinuous polyacrylamide gel electrophoresis (Disc-PAGE) under nonreducing and nondenaturing conditions (% separating gel, 4% stacking gel) and PAGE containing 0.1% sodium dodecyl sulfate (SDS-PAGE) were carried out according to the procedure of Hahm et al. (1983) in order to identify and characterize the tumor specific antigens. Immunoelectrophoresis (Graber and Williams, 1953) using the absorbed antisera specific for 3'-Me DAB induced rat hepatoma was performed on the test surface proteins of the plasma membranes of hepatomas induced by 3'-Me DAB, AAF, and DENA. Two dimensional gel electrophoresis (O'Farrell, 1975) was performed in order to identify the tumor specific antigens. Plasma membrane surface proteins of normal rat liver and rat hepatoma induced by 3'-Me DAB were subjected to first dimensional isoelectric focusing in tube gels under denaturing and reducing conditions. Focusing was done in the pH range of 4 to 9 using 1 mg protein each. Upon completion of isoelectric focusing, gel were placed on top of 10% polyacrylamide slab gels containing 0.1% SDS and second dimensional electrophoresis was performed in the vertical electrophoresis unit (Model 2001; LKB Bromma).

**Double Immunodiffusion:** Ouchterlony double immunodiffusion analysis (Ouchterlony, 1949) in 1% agarose (Agarose-M, LKB Bromma) was used to compare the plasma membrane surface proteins isolated from liver tumor cells induced by 3'-Me DAB, AAF, and DENA.

**Preparation of immunoaffinity column for identification of the tumor specific antigens:** Immunoglobulin G was separated from the absorbed antiserum using Protein A-Sepharose 6MB (Ery et al. 1978). Purified IgG in 0.5M NaCl, 0.1M sodium bicarbonate buffer pH 8.5 was coupled to cyanogen bromide-activated Separose CL-4B at the ratio of 7 mg/ml of packed beads (Choo et al. 1981). This column (1 x 10cm) was pre-eluted with 50ml M NaCl, 0.1M Tris-HCl buffer pH 8.6. The cell surface proteins from hepatoma induced by 3'-Me DAB were applied to the immunoaffinity column at a rate of 15 drops/min. After 15mg of the protein sample was loaded, the column was washed with 0.5M NaCl, 0.1M Tris-HCl pH 8.0 until the absorbance of the eluents at 280nm was less than 0.02. The column was then eluted with 150mM diethylamine pH 11.5. One ml fractions were collected, their absorbance measured 280nm and then they were immediately neutralized with 0.5M Tris-HCl buffer pH 7.6. Fractions of the protein peak were pooled and analyzed by SDS-PAGE.

**Purification of Tumor Specific Antigens:** Tumor specific antigens associated with rat hepatoma induced with 3'-Me DAB were differentiated and separated by gel permeation column chromatography using Sephadex G-200 (Pharmacia Fine Chemicals) and ion-exchange chromatography on a column of DEAE-Sephadex A 50 (Pharmacia Fine Chemicals).

**RESULTS**

Plasma membrane surface proteins were isolated from the livers of rats fed the control diet and the 3'-Me DAB containing diet at 3, 6, 8, 10, 12, 14 and 18
weeks of feeding, and analyzed by polyacrylamide slab gel electrophoresis. When the surface proteins were analyzed by 7% Disc-PAGE (Fig. 1), a new protein band (Rf=0.62) was identified from the surface proteins of tumor bearing rat liver. The band could not be seen in control protein samples (Fig. 1, Lanes 1 through 6); but it started appearing in the surface proteins obtained from rats fed with 3'-Me DAB even before the rat livers showed solid tumors (Lane 11). However, when the rats developed liver tumors, it was shown that the new protein band became heavy and more apparent (Fig. 1, Lane 11 through 16).

When the proteins were analyzed by 7% SDS-PAGE (Fig. 2), the differences between the control proteins (from normal rat livers; lanes 1 through 6, from livers of rats fed with 3'-Me DAB containing diet before developing tumors; lanes 7 through 10) and the plasma membrane surface proteins of rat hepatoma (lanes 11 through 16) could be recognized as several protein bands of which two protein bands (MW of approximately 66,000 and 73,000) were most apparent. Lane 17 shows the bands of molecular weight standards representing BSA (MW, 66,000) and ovalbumin (OVA; MW, 45,000), respectively.

To determine whether the antigens recognized by the absorbed specific antiserum for 3'-Me DAB are indeed located on the surface of the plasma membrane, the hepatomas induced by 3'-Me DAB were reacted with the absorbed antiserum, and the binding was monitored with goat anti-rabbit IgG conjugated to fluorescein. As is evident in Fig. 3, the plasma membrane of hepatoma (Fig. 3B) stains very brightly, suggesting that these antigens are indeed membrane surface-associated antigens.
Fig. 2. 7% SDS-PAGE analysis of plasma membrane surface proteins of rat liver cells. Lanes 1 through 16 contain identical samples as in Fig. 1 except that lane 17 shows the molecular weight standards; BSA, 66,000 and OVA, 45,000.

Fig. 3. Immunofluorescence staining patterns of normal rat hepatocyte (A) and rat hepatoma (B) induced by 3'-Me DAB (x 100). The plasma membrane of hepatoma (B) stains very brightly showing that the antigens recognized by the absorbed rabbit antiserum specific for 3'-Me DAB are on the membrane.
Fig. 4. Upper panel: Sephadex G-200 gel permeation column (0.9 x 56 cm) chromatography of the plasma membrane surface proteins (50 mg) separated from the normal rat liver cells (o-o) and rat hepatoma induced by 3'-Me DAB (●-●). The elution buffer was 1 mM EDTA-water, pH 7.4 and V₀ indicates the void volume of the column. Lower panel: Analysis of column fractions by 7% Disc-PAGE (A: control, B: 3'-MeDAB-induced hepatoma). Each lane represents the fraction directly above in the elution profile except that the far left lane shows each proteins before applying to the column. The tumor specific surface protein is indicated by an arrow.
Tumor Specific Antigens in Chemically Induced Hepatoma

Fig. 5. Analysis of the plasma membrane surface proteins by ion-exchange chromatography on DEAE Sephade X A-50 and SDS-PAGE. After gel filtration (Fig. 3), fractions (16-26ml) containing tumor specific antigen (or corresponding fractions in the case of control proteins) were pooled, concentrated and applied to the column (0.9 × 56 cm) of DEAE A-50. Upper panel shows the absorbancy profile of plasma membrane surface proteins of normal rat liver cells (○-○) and of rat hepatoma (●-●) eluted with step gradient formed by 0-0.5 M NaCl in 0.05 M Tris-Cl buffer, pH 7.4. Each fraction contained 3.0 ml. Lower panel shows 10% SDS-PAGE analysis of the column eluents. The numbers above gel lanes refer to the fraction number. A refers to the fraction of control proteins and B for proteins of 3’Me DAB induced hepatoma.
Fig. 4 compared the plasma membrane surface proteins of normal rat liver and 3′-Me DAB induced rat hepatoma by the elution on a column of Sephadex G-200 and showed the analysis of its fractions by 7% Disc-PAGE. The upper panel shows the elution profiles of the control proteins (−−−) and the proteins of 3′-Me DAB induced rat hepatoma (−−−). The result shows an almost identical elution pattern for both proteins except an increased protein peak for 3′-Me DAB induced hepatoma eluting in the inclusion volume (19-26ml). When analyzed were by 7% Disc-PAGE (Fig. 4, lower panel), the tumor associated protein was found to be localized in this peak area (19-26ml). The fractions of both proteins corresponding to this peak were pooled, concentrated, loaded on to a column of DEAE Sephadex A-50, and eluted with a step gradient formed with 0 to 0.4 M NaCl in 0.05 M Tris-Cl buffer, pH 8.3. The result (Fig. 5, upper panel) also showed nearly identical elution patterns for both proteins. However, when the column fractions were analyzed by 10% SDS-PAGE (Fig. 5, lower panel), the tumor specific antigen of 73K dalton was located in the peak including the fraction number 31, and the tumor specific antigen of 66K dalton was found in the last peak eluted with 0.4 M NaCl.

The plasma membrane surface proteins of normal rat liver and 3′-Me DAB induced rat hepatoma were also analyzed by two dimensional gel electrophoresis. From the result (Fig. 6), at least three tumor specific antigens which were not present in control proteins (Fig. 6, left panel) were identified (Fig. 6, right panel). They are a protein of 66K dalton (pl of 6.79) and two proteins of similar molecular weight (73K dalton) differing in their isoelectric pH (7.58 and 7.81).

From the above results, it is clearly demonstrated that there are at least three tumor specific antigens on the plasma membrane surface of rat hepatoma induced by the azo dye carcinogen, 3′-Me DAB.

With the above information, the following experiments were carried out to see whether any identical tumor specific antigens could be identified on the plasma membrane surface of rat liver tumors induced by various other known chemical hepatocarcinogens.

Fig. 7 shows the result of Ouchterlony double immunodiffusion analysis using the absorbed antiserum specific for 3′-Me DAB-induced rat hepatoma associated plasma membrane surface proteins. The result indicates that there are common tumor specific antigens on the plasma membrane surfaces of rat hepatomas induced by 3′-Me DAB and DENA judging by the continuing precipitin band. A similar result was also obtained (date not shown) with AAF indicating that all of the above three carcinogens cause the synthesis of common tumor specific antigen(s).

The immunoreactivities of the plasma membrane surface proteins of rat hepatomas induced by 3′-Me DAB, DENA, and AAF were also compared by im-
Tumor Specific Antigens in Chemically Induced Hepatoma

Fig. 7. Ouchterlony double immunodiffusion analysis of plasma membrane surface proteins isolated from rat liver cells. The absorbed rabbit antiserum (10µl) specific for 3'-MeDAB-induced hepatoma allowed to react by diffusion for 18 hrs at 25°C with the plasma membrane surface proteins (100µg) of rat liver cells in the outer wells. Well No. 1; normal rat liver cells, wells No. 2, 3, 5; 3'-Me DAB-induced rat hepatoma, Wells No. 4 and 6; DENA-induced rat hepatoma.

Fig. 8. Immunelectrophoresis pattern using the absorbed rabbit antiserum specific for 3'-Me DAB-induced hepatoma and plasma membrane surface proteins isolated from various carcinogen induced rat hepatoma. Proteins (150 µg each) in wells (well No. 1; control protein from normal rat liver cells, well No. 2; 3'-Me DAB-induced rat hepatoma, well No. 3; AAF-induced rat hepatoma, and well No. 4; DENA-induced rat hepatoma) were electrophoresed for 2 hrs at 100 V before the diffusion at 25°C for 18 hrs with the absorbed antiserum (100 µl) in troughs.

Fig. 9. Analysis of hepatoma-specific cell plasma membrane surface antigens eluted from immunoaffinity chromatography Lane A: MW standards, BSA (MW=66,000), OVA (MW=45,000), trypsinogen (MW=24,000), and lysozyme (MW=14,300), lane B, C: total cell plasma membrane surface proteins (100µg) of normal rat liver (B) and rat hepatoma (C), lane D,E,F,G: hepatoma specific cell surface antigens (10, 25, 50, 100µg each) eluted from immunoaffinity chromatography.
Yoon Soo Kim et al.

Figure 10. DEAE Sephadex A-50 column chromatography. A 3ml sample from immunoaffinity column was applied on a column (2.5 x 10 cm) of DEAE Sephadex A-50 and eluted with a step gradient (–) formed by 0.0-0.4M NaCl in 0.05M Tris buffer, pH 8.3.

Figure 11. SDS polyacrylamide gel (10%) electrophoretic patterns of proteins of various fractions (38, 39, 40, 49, 50, 51, 59, 60) eluted from DEAE Sephadex A-50.

separated by this immunoaffinity chromatography showed two major bands at the site of MW 73,000 and 66,000 on the SDS-PAGE (Fig. 9). These eluted proteins were further purified by DEAE-Sepharose A50 ion-exchange chromatography (Fig. 10) and analyzed by SDS-PAGE (Fig. 11). The results were identical to Fig. 5.

From these results, it is concluded that there are common tumor specific antigens on the plasma membrane surfaces of rat hepatomas induced by different chemical carcinogens, 3'-Me DAB, DENA, and AAF.

DISCUSSION

Since butter yellow (DAB) was reported to have a carcinogenic action by Kiosita (1936), many studies have reported on the morphological changes of rat liver during carcinogenesis induced by DAB (Orr 1940; Edwards and White 1942; Opie 1944) and the similarities of liver tumors induced by DAB and its derivatives (Cortell 1947; Miller and Miller 1948). Cunningham et al. (1950) studied the early changes in the liver cells by 3'-Me DAB and Richardson and Nachtnebel (1951) reported on the frequencies of tumor incidences and metastasis. Farber (1956) compared early histological changes in rat livers induced by ethionine, AAF and 3'-Me DAB and reported that they induced similar histological changes and thus caused similar metabolic effects. It is also known (Miller 1970) that AAF, DENA, and 3'-Me DAB are the same type of chemical carcinogens in the sense that they all create carcinogenic electrophilic reactants (ultimate carcinogens) when metabolized. AAF metabolizes to yield the more carcinogenic N-hydroxy-2-acetylaminofluorene and DENA yields the carcinogenic electrophile, C9H4. Metabolism of 3'-Me DAB is also known to involve N-demethylation and N- and ring hydroxylation followed by conjugation with a sulfate moiety to yield a strong electrophilic ester.

Miller and Miller (1947) first reported that DAB, when metabolized, formed a covalent linkage with the proteins of rat hepatocytes. It was also reported (Sorof et al. 1970; Sorof and Young 1973) that the metabolic product of 3'-Me DAB induced the h2-5s azoprotein with a specific protein in rat liver whose molecular weight was 60,000-80,000. The specificity and the function of the protein binding with the metabolites of the carcinogens are shown by Maini and Sorof by presenting evidence of the receptor protein which carries the metabolites of 3'-Me DAB. It was also reported by Patel (1979 and 1981) that 3'-
Tumor Specific Antigens in Chemically Induced Hepatoma

Me DAB acts as a kind of inhibitor for nuclear RNA processing, and thus causing distortion of RNA processing and resulting in a hepatomas. Bebawi et al. (1970) have shown that 3'-Me DAB is a more potent carcinogen than other structurally related DAB's.

At present, malignant tumors are thought to be developed in six successive steps (Kotin, 1976). They are 1. reaction between chemical carcinogens and nucleic acids, 2. effective repairing, 3. production of abnormal proteins induced by alterations of the genome, 4. progression of malignant changes, 5. formation of a cell mass in order to develop a malignant tumor from transformed cells, and 6. the growth of the tumor after the formation of a mass.

The presence of tumor specific antigens on the plasma membrane of DAB-induced hepatoma (D23) was first reported by Baldwin et al. (1971). They identified tumor specific antigens on the surface of a hepatoma by the indirect immunofluorescent antibody technique and studied the changes of the protein antigens during malignant transformation. This tumor specific antigen induced by DAB was separated by DEAE-cellulose column chromatography and gradient centrifugation after digesting the hepatoma with papain and the molecular weight was reported to be about 55,000 (Baldwin and Glaves, 1971; Baldwin et al. 1973). This protein is smaller than the DENA-induced guinea-pig hepatoma specific antigen of MW 75,000-150,000 extracted with 3M KCl (Meltzer et al. 1971). The tumor cell antigen isolated from papain digestion may not be an intact molecule. Pelltis et al. (1976) separated two tumor specific antigens (MW 40,000 and 75,000) from the 3M KCl extract of methylcholanthrene-induced fibrosarcoma in C3H/Hej mice after 0.25% trypsin digestion. It has been suggested that in order to isolate plasma membrane surface proteins in their intact molecular forms, proteolytic enzymes or detergents should be avoided.

In the present study, tumor specific plasma membrane surface proteins induced by 3'-Me DAB, AAF, and DENA were isolated using 3M KCl, and analyzed by the combined techniques of Disc PAGE, SDS-PAGE, and two-dimensional gel electrophoresis. It was identified that at least three tumor specific proteins of which molecular weight are 66 kdal (pI=6.79) and 73 kdal (pI=7.58 and 7.81), are newly expressed. The tumor specificity of these proteins on 3'-Me DAB induced hepatoma were confirmed by double immunodiffusion, immunoelectrophoresis, and immunoaffinity chromatography. The localization of these proteins was confirmed by the immunofluorescent technique. These results also provide evidence that there are common tumor specific antigens on the plasma membrane surface of rat hepatomas induced by different chemical carcinogens.

The 3'-Me DAB-induced tumor specific antigens have been partially purified by gel filtration chromatography and ion-exchange chromatography, and the efforts to characterize the molecular properties of these proteins and to elucidate the biological functions of these proteins are being made in this laboratory.

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