ULTRASTRUCTURAL LOCALIZATION OF IMMUNO- REACTIVE FUCOSYLCERAMIDE IN NORMAL AND ADENOCARCINOMA CELLS

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The localization of fucosylceramide recognized by a monoclonal antibody (MAb), PC47H, was examined immunohistochemically using normal and cancerous tissues. Immunoreactive fucosylceramide was observed in pancreas, parathyroid, submandibular gland, placenta, ovary, intestine, kidney, heart, plasma cells, adenocarcinomas of various organs and multiple myeloma, but not in lymphocytes and in squamous cell carcinoma. The immunoreactive fucosylceramide was localized mainly in the rough endoplasmic reticulum (ER) and plasma membranes of these cells. Selective expression of fucosylceramide in particular organs and adenocarcinomas may reflect a role of fucosylceramide.

Many fucose-containing glycolipids have been found in human adenocarcinomas and it has been shown that changes in the constitution of the glycoconjugates are the most common phenomenon associated with neoplastic change (4). It has been reported that fucosylceramide, a kind of glycolipid, is expressed significantly in colonic cancer but very poorly in normal colonic mucosa (14).

Recently, one of the present authors established a mouse anti-fucosylceramide (FC) monoclonal antibody (NMAb), PC47H, using neutral glycolipids from human pancreatic cancer tissue as an immunogen (5). Using this antibody, it has recently been shown that immunoreactive FC is abundantly expressed in adenocarcinomas of the lung, but expressed to a far smaller extent in poorly differentiated adenocarcinomas (16). More recently, we found significantly higher expression of FC in plasma cells and myeloma cells, but not in resting lymphocytes (11).

We report hereon the localization of FC recognized

by MAb PC47H in normal and cancer cells. The exact localization of FC has not previously been elucidated at either the light or electron microscopic level mainly because of technical difficulty in obtaining a monoclonal antibody reactive with FC.

MATERIALS AND METHODS

Surgically removed cancerous tissues from lung, stomach, liver, gallbladder, sigmoid colon, uterus, pancreas, thyroid, ovary and breast, and their normal counterparts, were obtained from Saitama Medical Center, Saitama Medical School, and Fukujūji Hospital, Japan Anti-Tuberculosis Association. Several normal tissues including thyroid, thymus, tonsil and fetal pancreas were obtained at autopsy performed within one hr after death.

Immunohistochemical observation by light microscopy

The normal and cancerous tissues were fixed with periodate-L-lysine-paraformaldehyde (PLP) overnight at 4°C. After fixation, the tissues were immersed in 10–20% sucrose-phosphate-buffered saline (PBS) solution overnight at 4°C and finally snap-frozen with dry ice-acetone in O.C.T. compound (Miles Inc., Elkhart, USA). The frozen tissues were then cut into

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%- μ m-thick sections in a cryostat, mounted on poly-L-lysine-coated slides and dried by blowing with a hair drier. MAb PC47H was generated in BALB/c mice by immunization with glycolipids extracted from human pancreatic cancer tissues, and used as a primary antibody (13).

Immunoelectron microscopic observation

Indirect immunoperoxidase staining was performed using MAb PC47H and the various thinly cut tissue sections. Prior to incubation with MAb PC47H (10 μ g/ml) overnight at 4°C, the sections were blocked by incubating them with 5% bovine albumin for 60 min at room temperature. After the primary reaction, the sections were washed three times with PBS for 5 min, then reacted with peroxidase-labeled rabbit anti-mouse immunoglobulins (1 : 100 dilution) (Dakopatts) for 120 min at room temperature. After careful washing with PBS, the sections were post-fixed with 2.5% glutaraldehyde for 30 min at room temperature, and washed with PBS three times. The sections were then reacted with PBS containing 3,3'-diaminobenzidine (2.5 mg/ml) and 0.05% H₂O₂, post-fixed with 1% OsO₄, and embedded in Epon 812 resin in a gelatin capsule. Ultrathin sections were cut with an ultramicrotome (Reichert Ultracut) and examined with an electron microscope (JEOL, JEM-100 B and JEM-1200 EX) (10).

RESULTS

Immunohistochemical observation

Immunoreactive FC was recognized in various types of cell from several tissues and organs. Positive immunostaining was observed in plasma cells in the bone marrow, plasma cells of the stomach and small intestine, distal tubules of the kidney, bronchial epithelial cells, decidua cells of the placenta, theca cells of the ovary, epithelia of the amnion, acinar cells and ductal cells of the submandibular gland, ductal cells of the pancreas, muscle cells of the heart and chief cells of the parathyroid (Table 1 and Fig. 1).

In fetal pancreas, exocrine ductal cells were immunostained with MAb PC47H. Plasma cells in the adult bone marrow reacted strongly with MAb PC47H, but lymphocytes from adult lymph nodes were negative. Adult tonsillar lymphocytes and thymocytes were also negative for FC immunostaining.

As our preliminary immunohistochemistry experiments had shown that immunoreactive FC was present particularly in well differentiated adenocarcinomas of the lung and ovary, adenocarcinoma tissues from various organs were used in this study.

As shown in Table 1, FC was expressed in adenocarcinomas of several organs and in multiple myeloma, whereas it was not expressed in squamous cell carcinoma of the lung and in small cell carcinoma. FC was distributed in the cytoplasm in a granular pattern (Fig. 1).

Immunoelectron microscopic observation

Since fresh-frozen tissues from each organ were not available, several tissues including lung cancer, breast cancer and fetal pancreas were utilized in this study. FC was found in the rough endoplasmic reticulum (ER) and plasma membranes of plasma cells, acinar cells of the fetal pancreas and adenocarcinoma cells, but was not expressed in every rough ER and plasma membrane (Fig. 2). There was no difference in FC distribution between adenocarcinomas from different organs.

DISCUSSION

It is difficult to distinguish malignant tumors from benign ones by biochemical methods. Therefore, much emphasis is placed upon morphology in cancer classification. However, many researchers have attempted to identify substances which are expressed to a greater extent than normal by cancerous tissues. Glycolipids may be potential candidates. The backbone of a sphingolipid, one kind of glycolipid, is sphingosine. Much attention has been paid to this substance because it is expressed abundantly in certain types of disease. In all sphingolipids, the amino group of sphingosine is acylated. A long-chain acyl CoA reacts with the sphingosine to form ceramide (N-acyl sphingosine), from which sphingomyelin, cerebroside, and gangliosides are derived. In Tay-Sachs disease, inherited as an autosomal recessive disorder, a large amount of ganglioside GM2 is deposited in the brain (12). Also, several ceramides containing carbohydrate moieties (fucosylated glycolipids) have been found in various human adenocarcinomas and their active sites have been identified immunochemically (1-3, 6).

FC has been detected in colonic cancer, but not in normal colonic mucosa, by biochemical methods (13). Other than this, no morphological studies of FC localization in cells and tissues have been reported. Interestingly, in this study FC was found to be localized mainly in the rough ER, with some in plasma membranes. Since the rough ER synthesizes proteins, but does not synthesize glycolipids, fucosylceramide, a kind of glycolipid, must be a constituent of the rough ER unit membrane and plasma membrane.

Various ceramide-containing carbohydrate

moieties have been recognized in several human cancer cell lines and human cancerous tissues by immunochemical techniques (1-3, 6). The cellular distribution of ceramide-containing sugars has been investigated, and it has been reported that CDw 17, CDw 60, CDw 65 and CD 77 react with lactosylceramide in granulocytes and platelets, NeuAc-NeuAc-Gal¹⁻⁴-(x)-ceramide in T lymphocytes, ceramide-dodecasaccharide 5c in granulocytes and glo-

botriosyl ceramide in B lymphocytes, respectively (7-9, 15). Since unique carbohydrate-containing ceramides are distributed in various tissues, they may be useful for cell classification. FC was expressed in plasma cells, but not in lymphocytes (11), and may therefore be a marker of B lymphocyte differentiation.

In summary, FC was distributed in normal cells as well as in cancer cells. The expression of immunoreactive FC in various organs and tissues is un-

TABLE 1. *Expression of immunoreactive fucosylceramide in various normal tissues and carcinoma tissues*

Tissue	Reactivity of MAb PC47H with
Tonsillar lymphocytes	—
Lymph node	—
Thymocytes	—
Pancreas	+ (acinar and ductal cells)
Thyroid	—
Lung	+ (epithelial cells)
Brain	—
Spinal cord	—
Kidney	+ (distal tubular cell)
Uterine cervix	—
Bone marrow	+ (plasma cell)
Placenta	+ (decidua cell)
Submandibular gland	+ (ductal and acinar cell)
Adrenal	—
Umbilical cord	+
Ovary	+ (theca cell)
Heart muscle	+
Amniotic membrane	+ (epithelial cell)
Testis	—
Liver	—
Stomach	+ (plasma cell)
Spleen	—
Parathyroid	+ (chief cell)
Jejunum	+ (plasma cell)
Sigmoid colon	—
Gallbladder ca.	+
Adenocarcinoma of the lung	+
Squamous cell ca. of the lung	—
Small cell ca. of the lung	—
Breast ca.	+
Sigmoid colon ca.	+
Stomach ca.	+
Thyroid ca.	+
Uterine cervix ca.	—
Multiple myeloma	+

The intensity of immunostaining was classified as follows: + tissue or cells immunostained with MAb PC47H; — not a single cell immunostained.

ca. = carcinoma

FIGS. 1A-E. Expression of immunoreactive fucosylceramide in multiple myeloma (A, $\times 165$), adenocarcinoma of the lung (B, $\times 165$), heart (C, $\times 200$), kidney (D, $\times 200$) and submandibular gland (E, $\times 200$). ABC-PO method. Counterstain with hematoxylin. Fucosylceramide is recognizable in cytoplasm in a granular pattern.

FIGS. 2A-C. Immunoelectron microscopic distribution of fucosylceramide recognized by MAb PC47H in fetal pancreas (A, $\times 27,000$), a plasma cell found in the lung tissue (B, $\times 15,000$) and breast cancer (C, $\times 10,000$). Fucosylceramide is recognizable in several rough ER and plasma membrane ($\rightarrow$). ABC-PO method.

que and it seems closely related to differentiation in lung adenocarcinoma cells and B cells (11, 15). But it is also expressed in normal cells from various organs, the function of which must be further studied.

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