Cancer-associated Galactosyltransferase as a New Tumor Marker for Ovarian Clear Cell Carcinoma

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ABSTRACT

Serum cancer-associated galactosyltransferase antigen (caGT) was assayed in gynecological cancer patients by means of a GT-II-reactive monoclonal antibody (MAb 3872)-based immunoassay. Thirty-six of 47 (75%) ovarian cancer patients showed a significant elevation of caGT in serum above the cutoff level of 200 milliunits/ml (mean ± 2 SD) determined from normal controls. Particularly, serum caGT levels in eight of nine patients with ovarian clear cell carcinoma were above the cutoff value, and six of them gave more than 2000 milliunits/ml. Elevation of caGT in serum from pregnant women was also detected, and the level increased during the course of gestation. Immunohistochemical study revealed that not only various ovarian carcinoma cells in vivo and in vitro, but also syncytiotrophoblast of early gestational placenta, fetal tissues such as mucus-producing cells in the lower alimentary tract, and renal tubules at the 11th week of gestation were stained with MAB 3872, thus indicating its oncofetal character. Compared with CA-125, caGT showed a lower false-positive rate (10%) in benign gynecological diseases, thus indicating its oncofetal character. Compared with CA-125, caGT showed a lower false-positive rate (10%) in benign gynecological diseases, and there was no correlation between caGT and CA-125 values. Therefore, caGT will be a useful tumor marker for ovarian cancers, especially for clear cell carcinoma.

INTRODUCTION

Recent interest in cancer-associated antigens has been focused on the aberrant glycosylation of glycoproteins and glycolipids (1), and the participation of glycosyltransferases has also been suggested in the synthetic process giving rise to these altered glycosylation patterns (2). However, the quantitative and qualitative changes in glycosyltransferases in cancers are still under investigation (3). GT in serum was reported as a useful prognostic marker in various cancers (4, 5) including ovarian cancers (6, 7), but the elevation of GT was also observed in inflammation, regenerative processes, and hepatic diseases (8, 9), indicating that an elevated GT level is not specific to cancer. Since Podolsky and coworkers (10-15) reported the presence of an isoenzyme of GT, or GT-II, in serum from patients with various malignant diseases, GT-II as a cancer marker has been studied intensively (16). Recently, Uemura et al. (17) established a monoclonal antibody that reacts with GT-II and were successful in applying it to the quantification of caGT in serum.

This paper presents the results of both the serum assay and immunohistochemical studies on caGT in gynecological tumors. The change in caGT level in the serum of pregnant women during the course of gestation and the immunohistochemical staining of fetus and placenta were also studied in order to investigate whether the expression of caGT is regulated oncodevelopmentally.

MATERIALS AND METHODS

Serum Samples. Serum samples were obtained from 225 patients who visited Keio University Hospital with various gynecological diseases such as benign ovarian tumor, ovarian tumor of low potential malignancy, ovarian cancer, uterine cervical cancer, uterine endometrial cancer, and uterine myoma, or with other diseases such as hepatitis, cirrhosis, hepatocellular carcinoma, and pancreatic cancer. Ovarian cancers were classified as serous, mucinous, clear cell (mesonephroid), endometrioid, undifferentiated, and Krukenberg types according to the WHO classification. Sera from healthy controls and pregnant women were collected from volunteers, and caGT levels of umbilical artery and vein and amniotic fluid were also measured. All samples were stored at —80°C before use.

Assay of GT Activity, caGT Value, and CA-125. GT activity was measured using UDP-[3H]galactose as donor and ovalbumin as acceptor. Ten μl of serum sample and 65 μl of GT assay mixture [10 μl of 2 mM UDP-[3H]galactose (New England Nuclear, Boston, MA) containing approximately 106 cpm, 5 μl of 0.2 mM manganous chloride, 2 mg ovalbumin, and 50 μl of CKT buffer (20 mM sodium cacodylate, 0.15 M potassium chloride, and 0.01% Triton X-100, pH 7.3)] were mixed. After incubation of the reaction mixture at 37°C for 3 h, the reaction was stopped by cooling in an ice bath. Then, a 50-μl aliquot was removed and spotted on 1-inch squares of Whatman 3MM paper and run with each set of assays in order to standardize all of the serum results. One unit of GT activity corresponded to 1 nmol of galactose transferred to ovalbumin/h at 37°C.

All assays were run in duplicate, and the mean value was reported. The coefficients of variation of inter- and intraassays were less than 10%.

caGT was specifically assayed by first selectively removing caGT from serum by immunoadsorption using immobilized MAB 3872, a monoclonal antibody that reacts with GT-II (17), followed by the assay of bound GT activity. In brief, purified MAB 3872 was covalently immobilized to 1,1'-carbonyldiimidazole-activated Trisacryl gel GF-2000 (Pierce Chemical Co., Rockford, IL) following the procedures suggested by the suppliers. Next, 100 μl of serum sample were combined with 30 μl of MAB 3872-coupled GF-2000 suspension, and the mixture was incubated at 4°C overnight with shaking. Then the gel was washed twice with 2.5 ml of cold (4°C) CKT buffer, and 75 μl of GT assay mixture (10 μl of 200 μM UDP-[3H]galactose containing approximately 5 × 105 cpm, 5 μl of 0.2 mM manganous chloride, 2 mg ovalbumin, and 60 μl of CKT buffer) were added. The GT activity assay was then continued as described above. This caGT immunoassay was linear with the amount of added GT-II and with time of incubation at 37°C (data not shown). A partially purified sample of GT-II was used as a reference control standard to normalize all patients' data. The caGT immunoassay could readily detect and quantify 10 milliunits/ml of serum (using 0.1 ml of serum for assay). All assays were run in duplicate, and the mean value was reported. The inter- and intraassay coefficients of variation were 10–15 and 8–12%, respectively.

CA-125 was assayed by radioimmunoassay kit (Centocor, Malvern, PA), and the cutoff value was set at 35 units/ml.

Immunoperoxidase Staining of Tissue Sections and Cell Lines. Tissue sections prepared according to the acetone-methanolbenzoate-xylene method and cultured cells fixed with acetone were stained by MAB...
3872 in combination with avidin-biotin-peroxidase complex reagents (Vector Laboratories, Inc., Burlingame, CA). Briefly, operation materials of 9 ovarian tumors (2 cases of serous cystadenocarcinoma, 1 clear cell carcinoma, 1 solid teratoma, 2 cases of undifferentiated cancer, 1 metastatic adenocarcinoma, 1 embryonal carcinoma, 1 mucinous cystadenoma of low potential malignancy), 3 cases of uterine cervical epithroid cancers, 1 uterine cervical adenocarcinoma, 16 cases of endometrial adenocarcinoma, fetuses of 11 and 16 weeks of gestation, 5 cases of placenta of ~7–9 weeks of gestation, and 10 cases of full-term placenta were fixed with acetone and xylene for 30 min and embedded in paraffin at 58°C. Human cell lines established from uterine cervical epithroid cancers [SKG-I (18), SKG-II (19), SKG-IIIa and IIIb (20)], endometrial adenocarcinoma [SNG-M (21), SNG-II (22)], uterine leiomyosarcoma [SKN (23)], ovarian rhabdomyosarcoma [RKN (24)], choriocarcinoma [NJG (25)], and ovarian clear cell carcinoma [RMG-I (26)], all of which were established in our laboratory, were grown in Ham's F-12 medium supplemented with 10% fetal calf serum and were fixed with acetone. Thin sections of tissue specimens and cultured cells were incubated with MAb 3872 and subsequently treated with biotinylated goat anti-mouse IgG antibody and avidin-biotin complex reagents (Vector Laboratories), followed by immersion in 3,3'-diaminobenzidine tetrahydrochloride solution (27).

RESULTS

GT Activity in Sera of Patients with Various Conditions. GT activity and its positive rate in sera of patients with various conditions are shown in Fig. 1. When the cutoff value was set at 168 units/ml (mean ± 2 SD of healthy controls), the positive rate was high in uterine cancers (cervical epithroid cancer, 72%; cervical adenocarcinoma, 73%; endometrial adenocarcinoma, 87%) and ovarian cancers (96%). However, 66% of the cases of benign uterine and ovarian tumors were also positive and sera from one-half of the cases with hepatitis or cirrhosis showed values over the cutoff level.

cαGT Value in Sera of Patients with Various Conditions. cαGT values for sera from patients with various conditions are summarized in Fig. 2. When the cutoff was set at 200 milliunits/ml (mean ± 2 SD), the positive rate of healthy controls was 1% (1 of 87), and that of gynecological benign diseases, including ovarian cyst and uterine myoma, and benign liver diseases, including hepatitis and cirrhosis, was 10% (4 of 40) and 0% (0 of 14), respectively. The positive rate of cαGT for malignant tumors was the highest for ovarian cancers (75%, 35 of 47), and that for other cancers was between 14 and 40%: uterine cervical epidermoid cancer, 30% (14 of 46); uterine cervical adenocarcinoma, 20% (3 of 15); uterine endometrial adenocarcinoma, 14% (6 of 44); and hepatoma, 40% (4 of 10). Fig. 3 indicates cαGT levels in the sera of benign, low potential malignant, and malignant ovarian tumors of various histological types. In benign and low potential malignant cases, the positive rate was 12 and 14%, respectively, however, except for low levels of cαGT in sera from patients with mucinous cystadenocarcinoma (22%, 2 of 9), a significant elevation was observed in sera from all other ovarian cancer patients: serous cystadenocarcinoma, 91% (10 of 11); clear cell carcinoma, 89% (8 of 9); endometrioid cystadenocarcinoma, 100% (5 of 5); and metastatic carcinoma, 80% (4 of 5). In addition, the cαGT value was over 2000 milliunits/ml in 6 of 8 positive cases of clear cell carcinoma. Fig. 4a indicates cαGT values in sera of patients at various clinical stages: the data show that cαGT tends to rise in pro-
Fig. 6. Immunohistochemical staining of ovarian tumors in vivo and in vitro with monoclonal antibody that reacts with GT-II (MAb 3872). Acetone-fixed tissue specimens or culture cells were stained by the avidin-biotin-peroxidase complex method. a, intravacuolar substances of clear cell carcinoma were positive (arrowheads). × 100. b, intracytoplasmic mucin of ovarian mucinous cystadenoma of low potential malignancy was positive (arrowheads). × 100. c, cell surface glycocalyx of ovarian serous cystadenocarcinoma was positive (arrowheads). × 200. d, intracytoplasmic areas of ovarian serous cystadenocarcinoma were stained (arrowheads). × 100. e, among 9 cell lines, a few cells of only the RMG-I cell line, which was established from an ovarian clear cell carcinoma, were positive for caGT (arrowheads). × 200. f, in a 16-week-old fetus, mucus-producing cells in the lower alimentary tract were stained (arrowheads). × 100. g, immunohistochemical staining of fetus with MAb 3872. In a 16-week-old fetus, renal tubules were positive (arrowheads). × 100. h, syncytiotrophoblasts of early gestational placenta were positive (arrowheads). × 100.
portion to the advance of the disease and that all of the recurrent cases had significantly elevated caGT. Fig. 4b shows the distribution of caGT according to tumor size, indicating that the larger the size of tumor, the higher, the caGT serum level.

Lack of Correlation between caGT Value and CA-125 in Ovarian Cancers. Levels of caGT and CA-125 were assayed simultaneously in serum from 22 patients with malignant ovarian tumors, and no significant correlation was recognized between the two (r = 0.217, P < 0.05).

GT Activity and caGT Value in Pregnancy. As shown in Fig. 5, both GT and caGT levels in sera of pregnant women increased as the gestational week advanced, but the rate of increase was steeper for caGT than for GT.

Table 1 shows the caGT values in umbilical vein and umbilical artery together with the level in the mother’s vein at full-term delivery. Compared with the caGT level in the mother’s vein, that in the umbilical vein and artery had a tendency to be higher.

**Immunoperoxidase Staining of caGT.** Among 29 cases of gynecological tumors, only 3 cases were positive for caGT; intravacuolar substances of clear cell carcinoma cells (Fig. 6a), intracytoplasmic mucin of mucinous cystadenoma cells of low potential malignancy (Fig. 6b), and cell surface glycolcalyx (Fig. 6c) and intracytoplasmic area (Fig. 6d) of serous cystadenocarcinoma cells were stained. Among 9 cell lines, a few cells only of the RMG-I cell line (Fig. 6e), which was established from an ovarian clear cell carcinoma, showed a positive intracytoplasmic reaction. In the fetus, mucus-producing cells in the lower alimentary tract (Fig. 6f) and renal tubules of the kidney (Fig. 6g) were stained. The syncytiotrophoblast was positive in all of 5 early gestational placentas (Fig. 6h), but none of 10 full-term placentas were stained.

**DISCUSSION**

Establishment of two new assay systems permitted us to determine serum GT activity and the caGT value in normal as well as in ovarian and uterine tumor-bearing individuals. As a result, GT activity tended to show a higher value in sera of patients with uterine cervical, endometrial, and ovarian cancers than in sera of healthy controls. As the elevation of GT activity in both sera and tissues has been reported previously by many authors (4-7), the present data are very understandable. However, as high GT activity with this assay system has often been recognized in sera from patients with benign gynecological diseases, such as uterine myoma and benign ovarian tumor, GT activity has not always proved to be specific for ovarian cancers. Meanwhile, Podolsky et al. (10-15) reported that GT in human sera is composed of two isoenzymes, GT-I and GT-II, that GT-I is responsible for most of the GT activity in normal serum, and that GT-II occasionally appears in sera of cancer patients. However, until now no assay system specific for GT-II has been reported, so the correct evaluation of GT-II as a tumor marker has not been fully investigated. Recently, Uemura et al. (17) succeeded in developing a caGT immunoassay using a newly produced monoclonal antibody which reacts with GT-II. Although the precise relationship between GT-II and caGT has not yet been fully clarified, use of this immunoassay in the present study proved that caGT was positive in only 10% of the cases of benign gynecological tumors, but in 75% cases of ovarian cancers, indicating that caGT is far more specific to malignancy than GT activity. The most noteworthy fact is that the caGT value in clear cell carcinoma was over the cutoff value in 8 of 9 cases and was very high in 6 of the 8 positive cases, thus suggesting the possibility that caGT could be used to presume the histological type of ovarian cancer. The assay of this isozyme may supplement the weak point of the gynecolog-

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* ND: not determined.
early pregnancy was strongly positive for caGT, indicating that they were positive, indicating that caGT has a tendency to lose its GT works as a recognition substance in various sociobiological functions on the cell surface (30-33). However, the positive possibility exists that some part of serum caGT of term gestation was stained by MAb 3872 in spite of the high serum staining of caGT in ovarian cancer tissues by using a monoclonal antibody that reacts with GT-II. As a result, ovarian tumor cells were clearly stained, suggesting that caGT produced by the tumor cells participated in the elevation of serum caGT.

A positive immunohistochemical reaction for caGT was observed on the intravacular substances of clear cell carcinoma cells, intracytoplasmic mucin of mucinous cystadenocarcinoma cells of low potential malignancy, and cell surface glycoalyx and intracytoplasmic areas of serous adenocarcinoma cells. As the true nature of intravacular substances of ovarian clear cell carcinoma has not yet been clearly identified, the meaning of the presence of caGT in them remains obscure, but this positive staining seems to correspond to the high positive rate of serum caGT in ovarian clear cell carcinomas. The localization of caGT on the mucin in ovarian mucinous cystadenocarcinoma cells of low potential malignancy is more understandable, because GT may play an important role in synthesizing mucin. A positive reaction for caGT in the intracytoplasmic areas and on the cell surface glycoalyx of serous cystadenocarcinoma cells also seems plausible, especially when we take into consideration the fact that caGT is one of the glycoprotein enzymes (17) and that GT works as a recognition substance in various sociobiological functions on the cell surface (30–33). However, the positive staining was observed only on cold acetone-fixed specimens, and none of the formalin-fixed, paraffin-embedded specimens were positive, indicating that caGT has a tendency to lose its antigenicity very easily. A most noteworthy fact is that the level of caGT in pregnant women increased during the course of pregnancy and reached its highest value at delivery. Immunohistochemical staining revealed that the syncytiotrophoblast of early pregnancy was strongly positive for caGT, indicating that the villi of early pregnancy may be the origin of serum caGT and that caGT may be expressed oncopvelopmentally (34). Strangely enough, however, none of the villi of 10 full-term placentas was stained by MAB 3872 in spite of the high serum value in pregnant women. Because the caGT values in umbilical artery and vein were higher than those in the mother’s vein, the possibility exists that some part of serum caGT of term gestational women comes from the fetus. The precise nature of caGT-producing cells present during pregnancy is a matter for further research. Finally, although the caGT immunoassay adopted in this study was sensitive enough to quantify the caGT level in serum, the protocol was too tedious to perform a large number of assays with satisfactory precision. Therefore, a more convenient immunoassay, such as the sandwich immunoassay, will be needed for further clinical application.

Recently, as Narihara et al. (35, 36) and Masri et al. (37) succeeded in cloning and sequencing of complementary DNA on April 13, 2017. © 1990 American Association for Cancer Research. cancerres.aacrjournals.org Downloaded from bovine, mouse and human N-acetylglucosamine (β 1→4) galactosyltransferase, respectively, the precise mechanism of cancer-associated abnormal gene expression of the enzyme is anticipated in the near future.

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