

Inverse Relationship of Expression between GM3 and Globo-Series Ganglioside in Human Renal Cell Carcinoma

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SAITO, S., NOJIRI, H., SATOH, M., ITO, A., OHYAMA, C. and ORIKASA, S. *Inverse Relationship of Expression between GM3 and Globo-Series Ganglioside in Human Renal Cell Carcinoma.* Tohoku J. Exp. Med., 2000, **190** (4), 271–278 — Renal cell carcinoma (RCC) is highly metastatic. We previously showed that expression of globo-series ganglioside is associated with the metastatic potential of RCC. However, the mechanism of metastasis remains largely unknown, and there is no effective therapy for metastasis. It was recently shown that induction of differentiation of colon cancer cells by brefeldin A was accompanied by an increase of GM3 with a concomitant decrease of neolacto-series gangliosides. To get a clue to a new method of therapy for RCC, we investigated whether the similar changes occur in RCC cells expressing globo-series ganglioside. Growth suppression and an increase of GM3 simultaneous with a decrease of monosialosyl galactosyl globoside, a member of globo-series gangliosides, were observed in human RCC cell line ACHN following brefeldin A treatment. The resultant change of the ganglioside profile is inversely related to the ganglioside pattern associated with the malignant potential of RCC and almost coincided with that representative of RCC cases showing favorable prognoses. It is suggested that the inverse relationship of expression between GM3 and globo-series ganglioside is reflected on the degree of malignancy of RCC, and may be useful as one of the indicators for exploiting treatment methods of RCC. ——— renal cell carcinoma; GM3; globo-series ganglioside; brefeldin A © 2000 Tohoku University Medical Press

Renal cell carcinoma (RCC) is a highly metastatic tumor. However, the mechanism of metastasis remains to be elucidated, and no effective therapy for metastasis has been developed (Ulchalker and Klein 1996; Mudlers et al. 1997). In general, solid malignant tumors are resistant to the conventional anti-cancer drugs. Among these, RCC is especially resistant to anti-cancer drugs and radiation, and only a few cases respond to immunotherapy such as interferon α and

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interleukin 2 (Mudlers et al. 1997). Even today surgery, only applied to the localized lesion, is the reliable method of treatment for RCC. Therefore, a new approach of therapy for RCC is urgently required.

In an attempt to get insight into the malignant potential of human RCC, we investigated changes of glycolipids (Saito et al. 1991) since glycolipids, especially gangliosides, are related to differentiation, carcinogenesis, and malignant progression (Hakomori 1996). In the studies on glycolipids, we found the relationship between expression of globo-series gangliosides and the metastatic potential of RCC (Saito et al. 1994, 1997; Satoh et al. 1996, 1999). It was anticipated that the gangliosides related to the malignant potential or the mechanism regulating the ganglioside synthesis could be targets of therapy for human RCC. Along this line, we have been searching for the therapeutic strategy using globo-series ganglioside as an indicator of malignancy. Recently, brefeldin A (BFA) was shown to induce differentiation and an increase of GM3 with a concomitant decrease of neolacto-series gangliosides in colon cancer cell line (Nojiri et al. 1999). GM3 is involved in differentiation of leukemia cell line HL60 (Nojiri et al. 1986), and also associated with decreased motility and apoptosis of tumor cells (Nojiri et al. 1999; Ono et al. 1999). On the other hand, neolacto-series gangliosides includes sialyl Lewis x, an adhesion molecule related to the metastatic potential of colorectal cancer (Hakomori 1996). Thus, BFA may have the differentiation-inducing action through changes of gangliosides (Nojiri et al. 1999). Induction of differentiation seemed to be a clue to a new method of treatment for RCC, and changes of gangliosides may underlie differentiation. Elucidating the relationship between differentiation and specific changes of gangliosides would lead to a new method of therapy for RCC. Therefore, we studied the changes of gangliosides in RCC cell line ACHN following BFA treatment in relation to the degree of malignancy or differentiation of RCC.

MATERIALS AND METHODS

Cell lines and culture

RCC cell line ACHN from a malignant pleural effusion was purchased from Dainihonsei-yaku Co. (Tokyo). ACHN highly expresses monosialosyl galactosyl globoside (MSGG), a member of globo-series gangliosides (Satoh et al. 1996) and was used for this study. ACHN cells were grown in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum (ICN Biomedicals, Inc., Aurora, OH, USA), 100 IU/ml penicillin and 100 μ g/ml streptomycin (Gibco BRL, Rockville, MD, USA), 10 mM HEPES (Dojindo, Kumamoto), and 2 mM L-glutamine (Gibco BRL) in a humidified atmosphere of 5% CO₂ in air.

BFA (Sigma, St. Louis, MO, USA) dissolved in 99.5% ethanol was added to the culture medium at the concentration of 0.05 μ g/ml and the final ethanol concentration of 0.1%. This concentration of BFA had no effect on cell viability of ACHN, and that of ethanol also had no effect on the cells, and an ethanol

control was not included in this study.

Cell proliferation assays were triplicated. Cells were counted with a hemocytometer, and viability of cells was assessed by a dye exclusion test with 0.3% trypan blue.

Monoclonal antibody

Monoclonal antibody RM1 (IgM) specific to MSGG was obtained as described previously (Saito et al. 1994).

Analysis of gangliosides

Packed cell pellet (0.5 ml) was extracted with 5 ml of isopropanol/hexane/water (55 : 25 : 20, v/v/v) twice, and then with 5 ml of chloroform / methanol (2 : 1, v/v) twice. Total extracts were combined and evaporated to dryness, and then subjected to Folch's partition. Upper phase separated by this partition was dialyzed against water, lyophilized, then subjected to diethylaminoethanol Sephadex A25 (Pharmacia, Uppsala, Sweden) column, and the ganglioside fraction was eluted as described previously (Saito et al. 1994). Ganglioside from human normal kidney or RCC tissue was extracted as described previously (Saito et al. 1994).

Thin layer chromatography (TLC) immunostaining was performed according to the modified version of Magnani et al. (1980). Gangliosides were applied on TLC plates for chromatography using a solvent system of chloroform/methanol/water (50 : 40 : 10, v/v/v) containing 0.05% CaCl₂. After drying, plates were blocked for 2 hours with 5% bovine serum albumin in phosphate buffer saline and reacted with culture supernatants of RM1 for 1 hour, incubated with biotinylated secondary antibody for 1 hour, incubated with avidin-biotin solution (Vector, Burlingame, CA, USA) for 30 minutes, and stained with 3', 3'-diaminobenzidine.

Reference glycolipids

GM3 was purchased from Wako (Osaka). GM2, GM1, and GD3 were purchased from Seikagaku-kogyo (Tokyo).

RESULTS

Proliferation of ACHN cells was suppressed (Fig. 1), and morphological changes were also observed following BFA treatment. In contrast to sheet-like growth pattern in non-treated cells, the treated cells gradually became disconnected from each other and took spherical appearance (Fig. 2).

Growth suppression was accompanied by an increase of GM3 and a concomitant decrease of MSGG in ACHN cells treated with BFA (Fig. 3). Increase of the band below GM3 corresponding to the level of GM2 was also observed 48 hours later. It was considered to be GM2, one of ganglio-series gangliosides, secondarily produced by way of increased GM3. With exception of an increase of this

ganglioside, the resultant change of ganglioside profile induced by BFA almost coincided with the ganglioside pattern typical of RCC cases showing favorable prognoses (Fig. 4). On the contrary, the ganglioside profile of untreated ACHN cells was similar to that representative of RCC cases showing unfavorable prognoses in that the long chain gangliosides increased (Fig. 4).

DISCUSSION

Recently, drugs inhibiting carbohydrate processing have been reported to decrease the malignant potential of cancer cells. Swainsonine, an indolizidine alkaloid and an inhibitor of Golgi α -mannosidase II, has been demonstrated to give objective improvements of symptoms and tumor burdens in advanced malignancies by blocking N-glycosylation processing (Goss et al. 1994). Tunicamycin, a natural antibiotics and also a N-glycosylation inhibitor, enhanced sensitivity of tumors cells to anti-cancer drug cisplatin (Noda et al. 1999).

Based on the study that BFA, a fungal metabolite, inhibits de novo globo- and neolacto-series glycolipid biosynthesis (Sherwood and Holmes 1992), colon carcinoma cell line was treated with BFA, focused on changes of gangliosides. Cancer cells were induced to differentiation and eventually led to apoptosis by BFA. During differentiation, an increase of GM3 and a concomitant decrease of

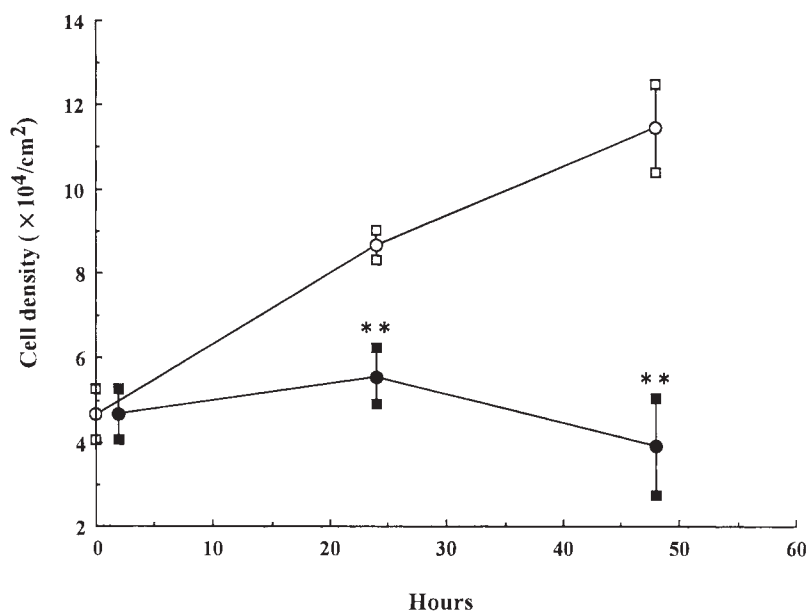


Fig. 1. Growth curves of ACHN cells treated with or without BFA. Proliferation of ACHN cells was suppressed by BFA. ** *, $p < 0.01$; \circ , - BFA; \bullet , + BFA

Fig. 2. Morphological changes of ACHN cells treated with or without BFA. a, ACHN cells just before treatment with BFA; b, ACHN cells after 24 hours of non-treatment; c, ACHN cells after 24 hours of treatment with BFA. With growth suppression, cells became disconnected from each other and took spherical appearance. Scale bar = 100 μm .

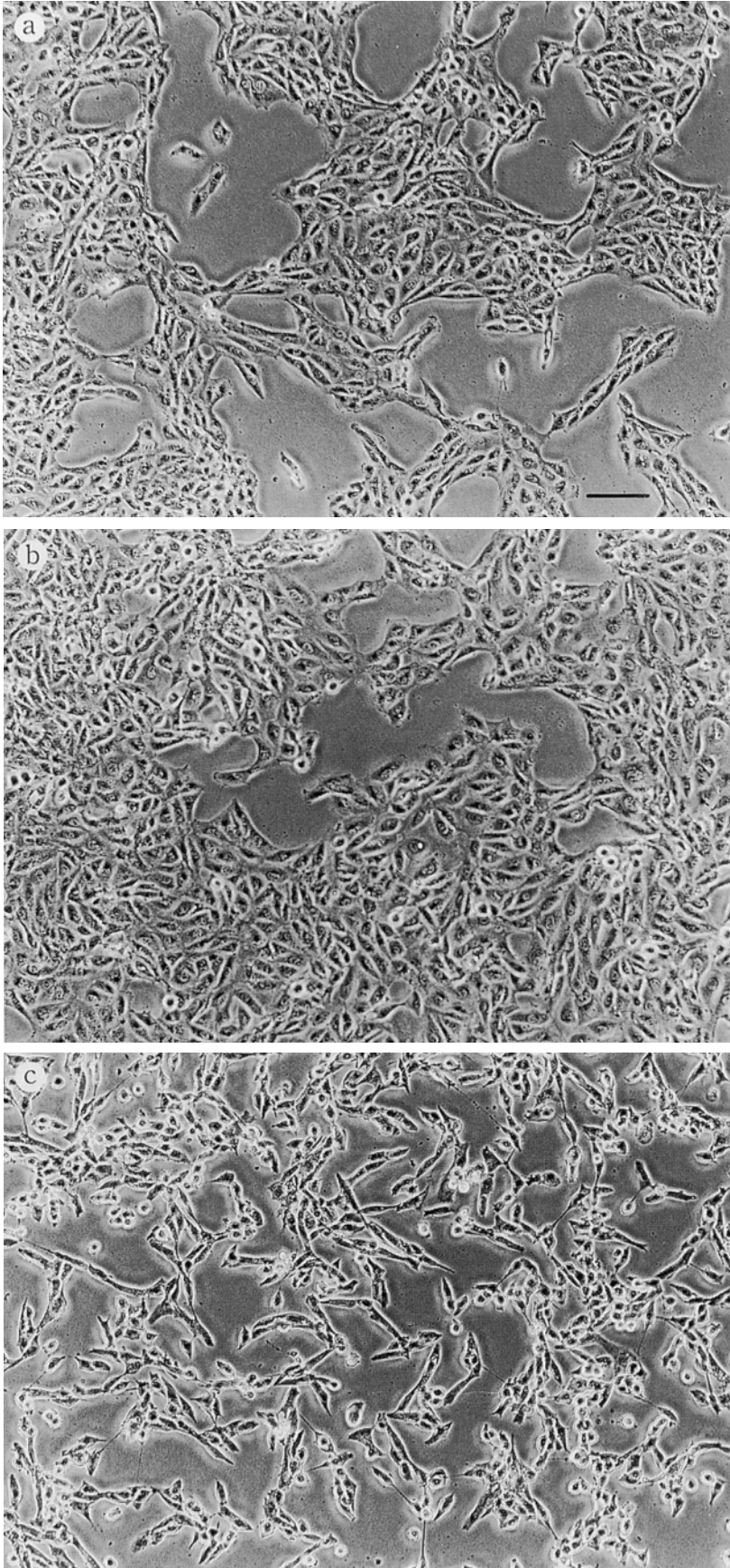


Fig. 2.

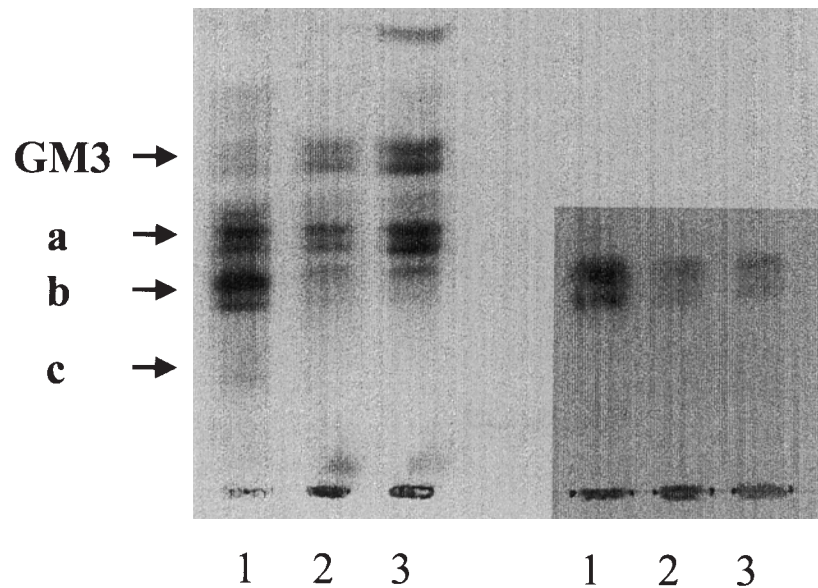


Fig. 3. Changes of the ganglioside profile in ACHN cells treated with BFA. Left panel, orcinol-sulfuric acid staining; right panel, immunostaining by anti-MSGG mAb RM1. a, the band corresponding to the level of GM2; b, those of GM1 and MSGG; c, that of DSGG (disialosyl galactosyl globoside). 1, the ganglioside profile in ACHN cells untreated; 2 and 3, those after 24 and 48 hours of treatment with BFA, respectively.

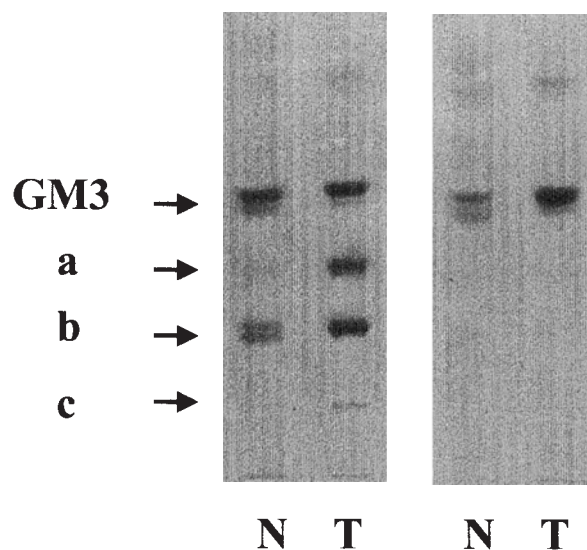


Fig. 4. The ganglioside patterns representative of RCC cases showing either favorable or unfavorable prognoses. Left panel, the ganglioside pattern typical of the cases that showed metastases soon after surgery for the primary tumors. Long chain gangliosides increased in the tumor tissue. Globo-series gangliosides were later found to be the major components of the long chain gangliosides (Saito et al. 1994). Right panel, the ganglioside pattern typical of the cases that showed favorable prognoses. Only GM3 increased and long chain gangliosides were hardly recognized in the tumor tissue. This case showed no evidence of recurrence for more than 10 years after surgery. N, normal kidney; T, primary tumor. a, the band corresponding to the level of GM2; b, those of GM1 and MSGG; c, that of DSGG.

neolacto-series gangliosides were observed (Nojiri et al. 1999). In this report, BFA treatment of ACHN cells gave rise to inhibition of cell proliferation, and to synchronous induction of an increase of GM3 and a concomitant decrease of MSGG. The band corresponding to GM2 increased after 48 hours whereas that decreased after 24 hours following BFA treatment. These phenomena may be interpreted to mean that the synthesis of ganglio-series gangliosides was also suppressed transiently by BFA and then activated due to precursor (GM3) accumulation. The resultant change of ganglioside profile induced by BFA almost coincided with the ganglioside pattern representative of RCC cases showing favorable prognoses (Saito et al. 1991). On the other hand, the ganglioside profile of untreated ACHN cells was similar to that typical of RCC cases showing unfavorable prognoses. In the previous study, MSGG was shown to be associated with the metastatic potential of RCC, and high nuclear grade was observed only in the cases positive to immunostaining of globo-series gangliosides but in none of the cases negative to the immunostaining (Saito et al. 1997). Furthermore, GM3 has been known to be a differentiation-inducer (Nojiri et al. 1986) and also an apoptosis-associated factor (Nojiri et al. 1999; Ono et al. 1999). In embryonal carcinoma cells, synthesis of glycolipid core chain structures changes from globo- to lacto-, and eventually to ganglio-series structures upon differentiation (Fenderson et al. 1990). From the previous studies and this report, a decrease of MSGG and an increase of GM3 synchronous with suppression of proliferation by BFA may imply differentiation of RCC cells. Thus, it is suggested that the inverse relationship of expression between GM3 and globo- or neolacto-series gangliosides is reflected on the degree of malignancy or differentiation of cancer cells, and may be useful as one of the indicators for exploiting cancer therapy.

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