Jpn. J. Cancer Res. 86, 361–367, April 1995

Enzymatic Basis for the Accumulation of Lewis^b Antigen in Uterine Endometrial Cancer

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In order to clarify the mechanism of the abnormal expression of Lewis^b antigen, which was specific for uterine endometrial cancer tissue, the activities of α1→2fucosyltransferase, α1→3fucosyltransferase, and α1→4fucosyltransferase in normal endometrial tissues and uterine endometrial cancer tissues were determined. Further, an immunocytochemical study of the expression of blood group-related carbohydrate antigens in 6 cultured cell lines derived from various gynecologic malignant tumors was performed and the α1→2fucosyltransferase, α1→3fucosyltransferase, and α1→4fucosyltransferase activities of these cell lines were determined. Compared with normal endometrium, uterine endometrial cancer tissues showed significantly higher values of α1→2fucosyltransferase, α1→3fucosyltransferase, and α1→4fucosyltransferase activities. The specifically strong expression of type I carbohydrate chains, particularly the Lewis^b antigen, was recognized in cultured cell lines derived from uterine endometrial cancer. Compared with those cell lines derived from uterine cervical cancer and ovarian cancer, the cultured cell lines derived from uterine endometrial cancer showed higher activities of α1→2fucosyltransferase and α1→4fucosyltransferase, which are enzymes related to the synthesis of Lewis^b antigen. The cell lines derived from uterine endometrial cancer showed specifically high values of α1→4fucosyltransferase activity. These results suggest that the α1→2fucosyltransferase and α1→4fucosyltransferase activities, especially the α1→4fucosyltransferase activity, contribute to the abnormal expression of the Lewis^b antigen in uterine endometrial cancer.

Key words: Fucosyltransferase — Uterine endometrial cancer — Blood group antigen — Le^b antigen

It has recently become evident that changes in glycoconjugates on cell surfaces are associated with malignant transformation of cells.[1)] It has also been reported that these changes are closely related to the biological properties of cancer cells, such as infiltration and metastasis, and analysis of abnormalities in the expression of glycolipids, including blood group-related antigens associated with malignant transformation of cells, have been performed on many organs.[2–9)] We have already studied abnormal expression of blood group-related antigens in uterine endometrial cancer, and found that the expression of type I carbohydrate chains, particularly the expression of Lewis^b antigens, was characteristic in uterine endometrial cancer.[10)] These results were different from those for several other cancers, e.g., in the stomach[2)] and lungs.[3)] However, the mechanism of abnormal expression of blood group-related antigens has not been clarified, because of difficulties in measuring the activity of fucosyltransferases, although it is assumed that the action of fucosyltransferases is involved. Therefore, in the present study, we measured the activity of α-fucosyltransferases in normal uterine endometrium and endometrial cancer as well as in cell lines derived from uterine endometrial cancer, ovarian cancer, and uterine cervical cancer, using a measurement system employing synthetic carbohydrate chains that are specific substrates of the various types of α-fucosyltransferases.[11–13)] We attempted to clarify the mechanism of abnormal expression of carbohydrate chains in uterine endometrial cancer with regard to the relation between the activity of individual α-fucosyltransferases and the expression of Lewis^b antigen, which is the antigen most specific for uterine endometrial cancer.

MATERIALS AND METHODS

Endometrial tissue Eight specimens of normal uterine endometrial tissues and 14 specimens of uterine endometrial cancer tissues were obtained from Keio University Hospital, Tokyo. They were perioperatively resected specimens.

Cultured cell lines derived from gynecologic malignant tumors We used the following uterine endometrial cancers: SNG-II,[14)] which was established in our laboratory, and Hec-108,[15)] kindly provided by Dr. Kuramoto (School of Medicine, Kitasato University, Kanagawa).

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The cell lines that were derived from uterine cervical epidermoid cancer were SKG-IIIa and SKG-IIIb,[16] and those derived from ovarian cancer were RMG-II[17] (clear cell adenocarcinoma) and RTSG[18] (poorly differentiated adenocarcinoma). All cell lines derived from uterine cervical epidermoid cancer and ovarian cancer were established in our laboratory. Using a closed incubator (37°C, 5% CO_2), the cells were cultured as static monolayers in 10% fetal calf serum (FCS) + kanamycin (25 μg/ml) + Ham's F-12 culture medium.

Monoclonal antibody MSN-1, a hybridoma producing mouse monoclonal antibody to Lewis^b, established in our laboratory,[19] was used as a Lewis^b probe. Mouse monoclonal antibodies which are specific for A, B, and H group antigens were purchased from DAKO Corporation (Glostrup, Denmark), while monoclonal antibody against Lewis^a was obtained from Green Cross Corporation (Osaka). Monoclonal antibodies against so-called Lewis^x and Lewis^y antigens[20] were provided by Dr. Hirohashi (Pathology Division, National Cancer Center Research Institute, Tokyo), and a monoclonal antibody against neolactotetraosyl ceramide (nLc4) antigen,[21] which reacts with the nonreducing terminal structure of type II chain, was donated by Prof. Hata (Department of Pathology, Keio University). A hybridoma producing human monoclonal antibody against lactotetraosyl ceramide (Lc4) HMST-1, was established in our laboratory.[22] The epitope recognized by HMST-1 was determined to be a lacto-series type I chain containing glycosphingolipid.[22]

Immunocytochemical staining Immunocytochemical staining was performed by the avidin-biotin complex method as previously reported.[10] Briefly, cells were incubated with the monoclonal antibody at room temperature for 1 h and subsequently treated with biotinylated horse anti-mouse IgG and avidin-biotin complex reagent (Vector Laboratory Inc., Burlingame, CA). The staining patterns were evaluated on the basis of both staining intensity and incidence of positive cells. The intensity was graded on an arbitrary scale as weak, moderate, or strong, and the incidence was classified into three grades, $<10\%$, 10% to 50%, and $>50\%$, according to the percentage of positive cells. By combining both the intensity and incidence, we classified the reactivity as (±), (+), (++) or (+++).[10]

α-Fucosyltransferase assay α-Fucosyltransferase activities in uterine endometrial tissues and in cultured cell lines were determined by the method of Yazawa and his colleagues.[11-13] Uterine endometrial tissues, and cultured cell lines derived from various gynecologic malignant tumors (1 g each) were each mixed with 2% Triton X-100 (Sigma, St. Louis, MO) containing PBS, and the mixtures were sonicated at 4°C. Centrifugation at 10,000 rpm for 30 min gave an enzyme solution, which was used for the measurement of the α1→2fucosyltransferase, α1→3fucosyltransferase, and α1→4fucosyltransferase activities.

RESULTS

α-Fucosyltransferase activities in uterine endometrial tissues (Fig. 1) In normal uterine endometrial tissues, α1→2fucosyltransferase, α1→3fucosyltransferase, and α1→4fucosyltransferase activities were 10.0±4.3, 7.2±

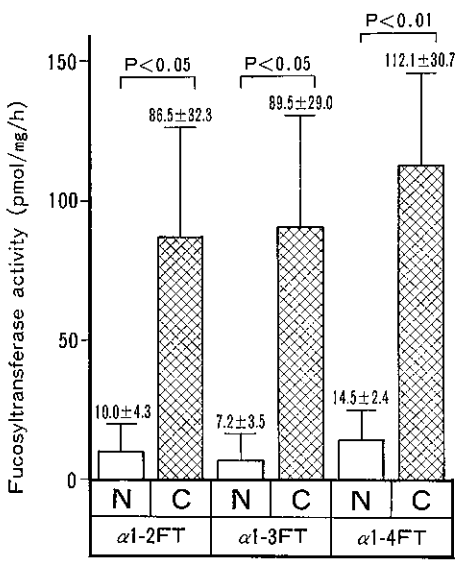

Fig. 1. α1→2Fucosyltransferase, α1→3fucosyltransferase, and α1→4fucosyltransferase activities in uterine endometrial tissues. Fucosyltransferase (FT) activities were measured as described in "Materials and Methods." N, normal endometrium (n=8); C, endometrial cancer (n=14).

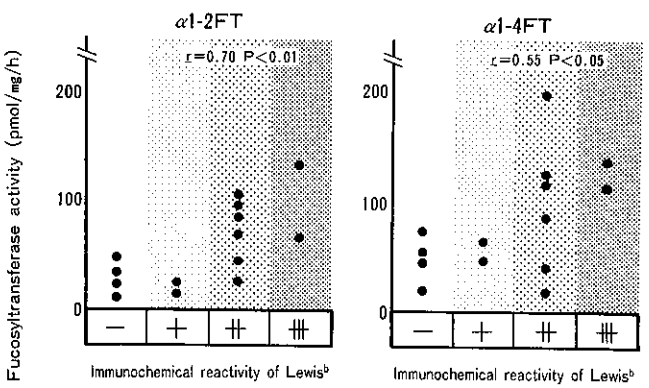

Fig. 2. Relationship between Lewis^b antigen expressed in endometrial cancers and α1→2fucosyltransferase and α1→4fucosyltransferase activities.

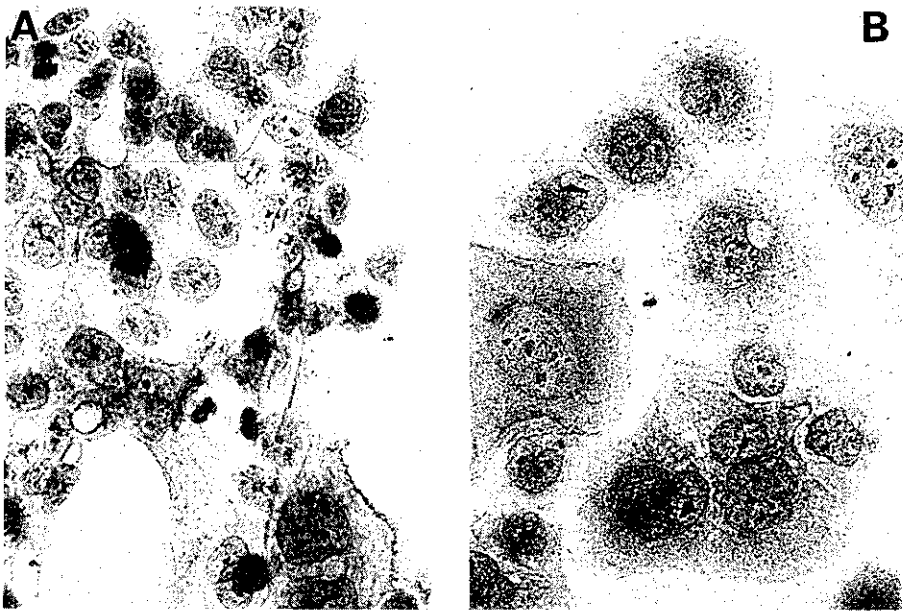

Fig. 3. Reactivity of anti Lewis^b antibody with cultured cell lines derived from uterine endometrial cancers. A, SNG-II; B, Hec 108; ×100.

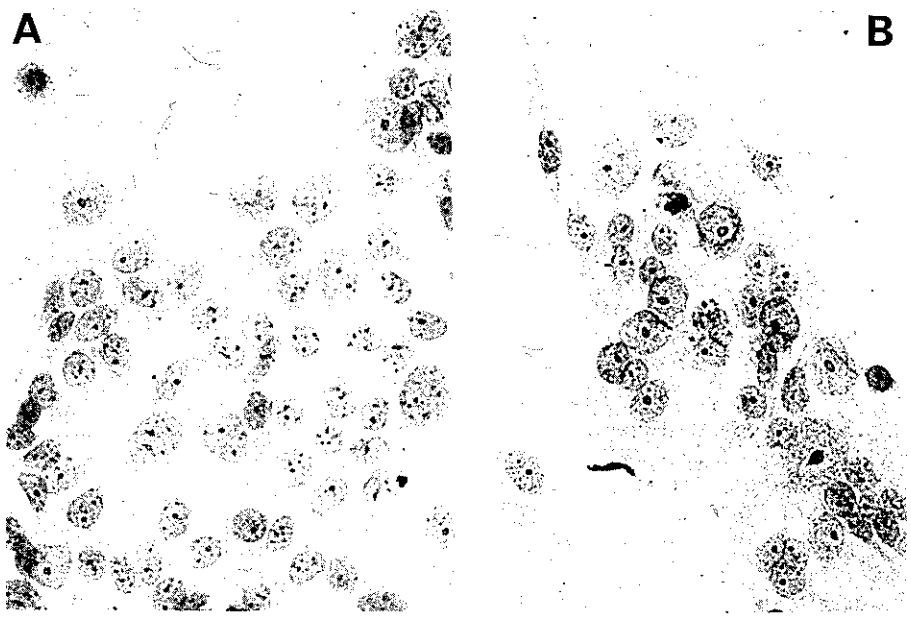

Fig. 4. Reactivity of anti Lewis^b antibody with cultured cell lines derived from uterine cervical epidermoid cancers. A, SKG-IIIa; B, SKG-IIIb; ×100.

3.5, and 14.5±2.4 pmol/mg/h, respectively. On the other hand, in uterine endometrial cancers, the activities were much higher, 86.5±32.3 ($P<0.05$), 89.5±29.0 ($P<0.05$), and 112.1±30.7 ($P<0.01$) pmol/mg/h, respectively. We also investigated the relationship between fucosyltransferase activities in endometrial cancers and expression of Lewis^b antigen. The activities of α1→2fucosyltransferase and α1→4fucosyltransferase in endometrial cancers were correlated positively with the expression of the Lewis^b antigen (Fig. 2). α1→3Fucosyltrans-

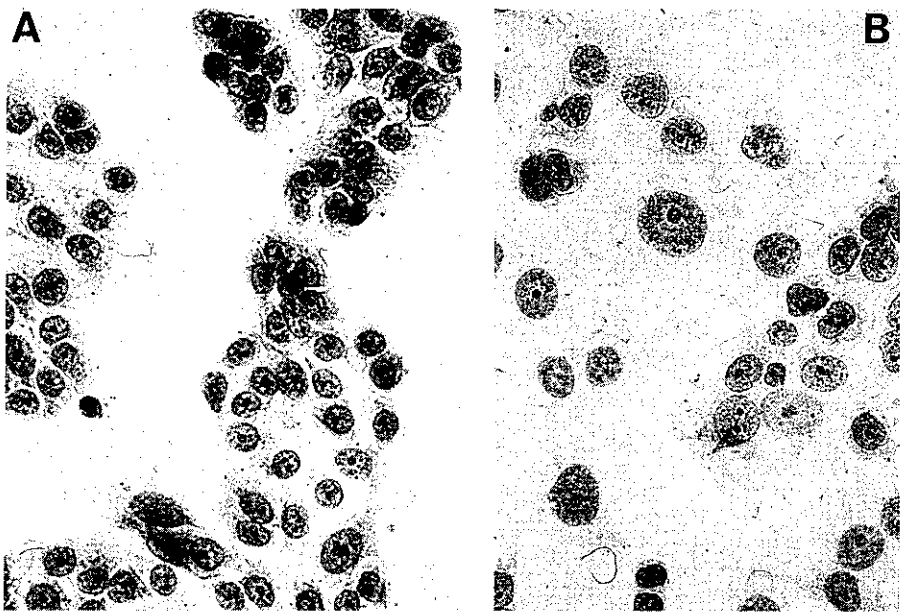

Fig. 5. Reactivity of anti Lewis^b antibody with cultured cell lines derived from ovarian cancers. A, RMG-II; B, RTSG; ×100.

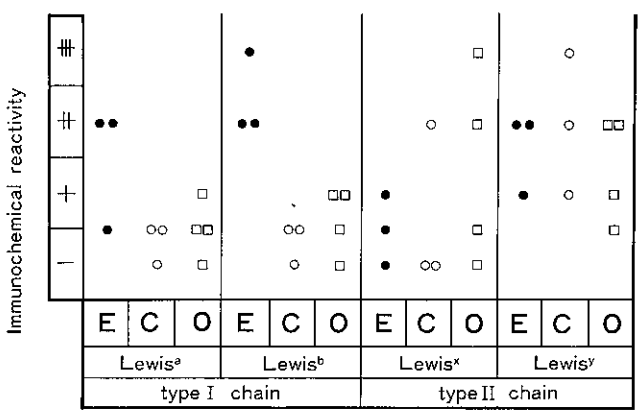

Fig. 6. Expression of blood group-related carbohydrate antigens in uterine endometrial cancer cell lines (E, ●), uterine cervical cancer cell lines (C, ○), and ovarian cancer cell lines (O, □). Immunocytochemistry was performed as described in "Materials and Methods."

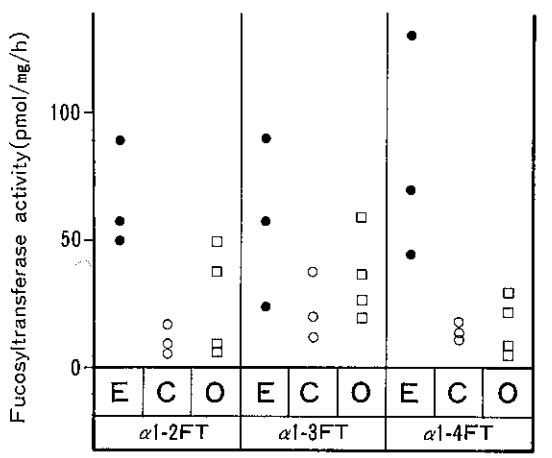

Fig. 7. α1→2Fucosyltransferase, α1→3fucosyltransferase, and α1→4fucosyltransferase activities in cultured cell lines derived from various gynecological cancers: uterine endometrial cancer cell lines (E, ●), uterine cervical cancer cell lines (C, ○), and ovarian cancer cell lines (O, □).

ferase activity, however, showed no definite correlation with this antigen (data not shown).

Expression of blood group-related antigens in cultured cell lines derived from various gynecologic malignant tumors Immunocytochemical staining of cultured cell lines with anti Lewis^b antibody is shown in Figs. 3–5. SNG-II and Hec108 were strongly positive (Fig. 3), whereas SKG-IIIa and SKG-IIIb were negative (Fig. 4). RMG-II was weakly positive, but RTSG was negative (Fig. 5). Among type I carbohydrate chains, Lewis^a and Lewis^b antigens definitely appeared on the cell lines derived from uterine endometrial cancer (Fig. 6). Lewis^b antigen was weakly expressed in the cell lines derived from uterine cervical and ovarian cancers, whereas expression of Lewis^b antigen was definite and specific for the cell lines derived from uterine endometrial cancer. The expression of type II carbohydrate chains such as

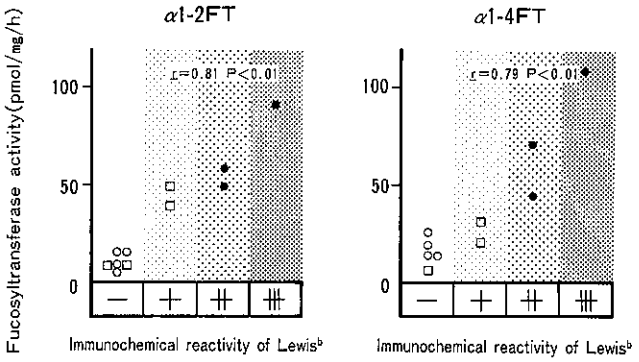

Fig. 8. Relationship of Lewis^b antigen expressed in cultured cell lines derived from various gynecological cancers to α1→2fucosyltransferase and α1→4fucosyltransferase activities of the cell lines: uterine endometrial cancer cell lines (●), uterine cervical cancer cell lines (○), and ovarian cancer cell lines (□).

Lewis^x and Lewis^y antigens was stronger than that of type I carbohydrate chains in the cell lines derived from uterine cervical cancer and ovarian cancer. There was no significant difference among the cell lines derived from uterine endometrial cancer, uterine cervical cancer, and ovarian cancer as to the expression of A, B, H, Lc4, and nLc4 antigens.

Activities of α-fucosyltransferases in the cultured cell lines derived from various gynecologic malignant tumors (Fig. 7) Compared with the cell lines derived from uterine cervical cencer and ovarian cancer, those derived from uterine endometrial cancer showed higher activities of α1→2fucosyltransferase and α1→4fucosyltransferase, which mediate Lewis^a and Lewis^b antigen synthesis. α1→2Fucosyltransferase and α1→4fucosyltransferase activities were low in the cell lines derived from uterine cervical and ovarian cancers, whereas the activity was particularly high in all the cell lines derived from uterine endometrial cancer. No significant difference was recognized among the 3 types of cell lines as to the activity of α1→3fucosyltransferase. Finally, the relationship of fucosyltransferase activities to Lewis^b antigen in the various cultured cell lines was investigated. The α1→2fucosyltransferase and α1→4fucosyltransferase activities in the cell lines were correlated positively with the expression of the Lewis^b antigen (Fig. 8), whereas the α1→3fucosyltransferase activity showed no clear correlation (data not shown).

DISCUSSION

α1→2Fucosyltransferase, α1→3fucosyltransferase, and α1→4fucosyltransferase are glycosyltransferases involved in the synthesis of H-type antigen and Lewis group antigens by catalyzing the addition of fucose to Lc4 or nLc4. H-type antigen is synthesized by the addition of a single fucose to Lc4 or nLc4, catalyzed by α1→2fucosyltransferase. The Lewis^a antigen is synthesized by addition of a single fucose to Lc4 by α1→4fucosyltransferase, and Lewis^b antigen is synthesized by addition of two fucoses to nLc4 by the concerted action of α1→2fucosyltransferase and α1→4fucosyltransferase. In the same way, fucose binds to nLc4 to synthesize Lewis^x type antigen, by the action of α1→3fucosyltransferase, or to form Lewis^y antigen by the concerted action of α1→2fucosyltransferase and α1→3fucosyltransferase. In our immunohistochemical study using monoclonal antibodies to blood group-related antigens, we found that the expression of fucosylated carbohydrate chains with fucose in the terminal position was increased and that the Lewis^b antigen showed the highest rate of expression in uterine endometrial cancer.[10] Therefore, we suggested that the expression of fucosylated carbohydrate chains, especially Lewis^b antigen, is specific to uterine endometrial cancer. It has been indicated that the mechanism of synthesis of aberrant blood group-related antigens involves changes in the activities of glycosyltransferases associated with malignant transformation. However, there have been few studies that directly clarify this relation, and no report has appeared concerning uterine endometrial cancer.

In the present study, the activities of α1→2fucosyltransferase, α1→3fucosyltransferase, and α1→4fucosyltransferase were all significantly higher in uterine endometrial cancerous tissue than in the normal uterine endometrial tissue, and cultured cell lines derived from uterine endometrial cancer showed higher α1→2fucosyltransferase and α1→4fucosyltransferase activities than cultured cell lines derived from ovarian and uterine cervical cancers. Therefore, the data suggest that the increase in fucosyltransferase activities is closely related to the expression of fucosylated carbohydrate chains in uterine endometrial cancer. We showed that the reactivity of anti Lewis^b antibody tends to be stronger in tissues of endometrial cancer with higher α1→2fucosyltransferase and α1→4fucosyltransferase activities, and we demonstrated that anti Lewis^b antibody reactivity also tends to be stronger in cultured cell lines with the higher α1→2fucosyltransferase and α1→4fucosyltransferase activities. Since it is clear that there is a positive correlation between the activities of these enzymes and anti Lewis^b antibody reactivity, a close relation is suggested between α1→2fucosyltransferase and α1→4fucosyltransferase activities and the expression of Lewis^b antigen, whose synthesis involves these enzymes.

Three cultured cell lines derived from uterine endometrial cancer showed high values of α1→4fucosyltransferase, and the expression of the type I carbohydrate chains was greater in the cultured cell lines derived from

uterine endometrial cancer than in those derived from ovarian and uterine cervical cancers, suggesting that not only the expression of the type I carbohydrate chain, but also an increase in α1→4fucosyltransferase activity is one of the characteristics of uterine endometrial cancer. It has been reported that distal colorectal tissue shows a similar pattern of expression of blood-group antigens to the endometrial tissue of the uterus and that the activity of α1→4fucosyltransferase associated with malignant transformation is also increased in the distal colorectal tissue.[11] In genetic studies on α-fucosyltransferase, five types of cDNAs have been reported.[23–27] FTIII is known to be the gene that codes for α1→4fucosyltransferase. It remains to be resolved whether the increased α1→4fucosyltransferase activity of uterine endometrial cancer described in this paper is caused by a change in the FTIII gene, or whether it is associated with a new cancer-associated gene. This point can be studied by using the cDNA of α1→4fucosyltransferase.

Recently, it has been pointed out that the sialylated Le^a or sialylated Le^x antigen expressed on the surface of cancer cells plays an important role in the metastasis of cancer cells by working as the ligand carbohydrate chain of selectin on endothelial cells.[28–31] This fact suggests that carbohydrate chains located on the cell surface have a close relationship with the specific function of cancer cells. In clinical and statistical examinations of endometrial cancers, we have obtained results indicating that the five-year survival rate is significantly lower in patients with uterine endometrial cancers that do not react with the monoclonal anti $Lewis^b$ antibody than in those whose cancer cells do react with anti $Lewis^b$ antibody (data not shown). Therefore, the specific expression of $Lewis^b$ antigen in uterine endometrial cancers is possibly related to the biological features of uterine endometrial cancer cells. Further examination of the role played by the fucosylated carbohydrate chains in uterine endometrial cancer is needed.

ACKNOWLEDGMENTS

The authors are very grateful to Dr. S. Hirohashi, Chief of The Pathology Division and Deputy President of the National Cancer Center Research Institute of Japan, and Prof. Hata, Department of Pathology, School of Medicine, Keio University, for providing us with monoclonal antibodies against $Lewis^x$, $Lewis^y$, and nLc4 antigens and for their helpful advice.

(Received September 22, 1994/Accepted January 18, 1995)

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