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Effect of MUC1 Mucin, an Anti-adhesion Molecule, on Tumor Cell Growth

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MUC1 mucin is expressed in a wide variety of tumors and is considered to function as an anti-adhesion molecule which inhibits cell-to-cell interactions. To reveal the biological significance of this activity in tumor cells, MUC1 cDNA was transfected into EJNIH3T3 cells and human colon cancer cell lines, CHCY1 and DLD1. The *in vivo* growth rate of MUC1$^+$ (MUC1-transfected) EJNIH3T3, CHCY1 and DLD1 cells in SCID mice was clearly lower than that of MUC1$^-$ (mock transfectant) cells. Several *in vitro* experiments using MUC1$^+$ EJNIH3T3 cells were performed to analyze the mechanisms for the decreased *in vivo* tumor growth. It was found that (i) the *in vitro* growth rate of MUC1$^+$ EJNIH3T3 cells was also decreased compared to that of MUC1$^-$ cells, (ii) the DNA synthesis of MUC1$^+$ EJNIH3T3 cells after stimulation with either growth factor (fetal calf serum or bombesin) or extracellular matrix (collagen or fibronectin) was lower than that of MUC1$^-$ cells, and (iii) MUC1$^+$ EJNIH3T3 cells grew more slowly than MUC1$^-$ cells on both collagen- and fibronectin-coated dishes. These data suggest that MUC1 mucin may regulate tumor cell growth through inhibition of cell-to-cell, growth factor-to-receptor and cell-to-matrix interactions.

Key words: MUC1 gene — Mucin — Anti-adhesion activity — Tumor cell growth — Growth regulation

MUC1 is a large, heavily glycosylated, type I transmembrane protein,[1-4] and is expressed in a wide variety of epithelial cells.[5] The characteristic feature of its primary structure is its extracellular domain, most of which consists of around 30 to 90 tandemly repeated sequences. One repeat contains 20 amino acids, being rich in serine, threonine and proline, and two *O*-glycosylation sites have been identified.[6] Although the expression of MUC1 is substantially increased in various tumors with high frequency,[7] its biological functions in them *in vivo* remain unknown. It has recently been shown that overexpression of MUC1 on the membrane of cultured cells inhibits their aggregation and interaction with cytotoxic lymphocytes, probably due to its large, extended and rigid structure.[8,9] These findings suggest that MUC1 may impinge on at least two important steps of the metastatic process, the release of cells from a tumor and their escape from immune surveillance.[8] The results described here indicate another possible important role of MUC1 in tumor cells: regulation of cell growth.

MATERIALS AND METHODS

Cell lines The cell lines used in this study were human colorectal carcinoma cell lines, CHCY1 and DLD1, and a mouse fibroblast cell line transformed by activated H-*ras* gene, EJNIH3T3, which were obtained from the Japanese Cancer Research Resources Bank. They were maintained in Dulbecco's modified Eagle's medium (D-MEM) (GIBCO-BRL, Grand Island, NY) supplemented with 200 mg/liter ampicillin, 100 mg/liter kanamycin and 10% (vol/vol) fetal calf serum (FCS).

Transfection of cultured cells The mammalian expression vector pHβAPr-Neo containing the full-length MUC1 cDNA and the vector containing antisense MUC1 cDNA were kindly provided by Dr. Metzger (Duke University). Liposome-DNA mixture was made up of 20 μg of the plasmid and 60 μl of Transfectace (GIBCO-BRL) in 6 ml of D-MEM. A mouse fibroblast cell line, EJNIH3T3, and human colorectal cancer cell lines, CHCY1 and DLD1, were washed twice with D-MEM and then incubated overnight with 6 ml of liposome-DNA mixture. Six milli liter of D-MEM supplemented with 20% FCS (vol/vol) was added, and the cells were incubated for another 72 h. G418 (Geneticin, GIBCO-BRL) was then added in the culture medium at the concentration of 800 μg/ml. The resultant transfectants were cloned by the limiting dilution method. The expression of MUC1 gene products on transfectants was confirmed by immunostaining with anti-MUC1 core protein monoclonal antibody (mAb) MUSE11 as previously described.[10,11] The cells transfected with the vector containing anti-sense MUC1 cDNA were used as mock transfectants.

Aggregation assays Aggregation assays were performed basically according to the method of Rojas *et al.*[12] The transfectants at the point of confluence were detached from the plastic surfaces by a 5-min incubation in D-MEM with 5 m*M* EDTA, and were washed with Puck's saline (5 m*M* KCl/140 m*M* NaCl/8 m*M* $NaHCO_3$, pH

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7.4) 3 times and pushed through a 27 gauge needle 3 times to make single cell suspensions. Triplicate aliquots (1×10^5 cells/ml of Puck's saline with 0.8% FCS and 50 U/ml deoxyribonuclease I (Pharmacia, Uppsala, Sweden) with either 10 m*M* Ca^{2+} or 0 m*M* Ca^{2+}) of the single cell suspension were seeded in 48-well cell culture plates (Costar, Cambridge, MA) and shaken in a circular motion at a frequency of 70 rpm at 37°C. The number of single cells was counted at 0, 1 and 2 h after shaking using a hemocytometer and the ratio of aggregated cells was calculated.

Tumor assay in SCID mouse The transfectants were detached from the plastic surfaces by a 5-min incubation in D-MEM with 5 m*M* EDTA, washed twice with D-MEM, and pushed through a 27 gauge needle 3 times to make single cell suspensions. Then 2×10^5 or 2×10^6 cells was inoculated subcutaneously into female SCID mice (6 weeks) in a volume of 100 ml into the right or left flank (Fox Chase SCID mouse, Nippon CLEAR, Tokyo). Mice bearing tumors were killed at 8 weeks after inoculation. Tumors were surgically resected and a post-mortem examination was carried out. Each primary tumor was weighed, fixed in neutral buffered formalin and embedded in paraffin. Multiple sections of tumors were stained with hematoxylin and eosin for morphological examination.

Flow cytometry Unpermeabilized transfectants were incubated with mAb MUSE11 for 30 min at 4°C. They were washed 3 times with cold phosphate-buffered saline (PBS) and then incubated with fluorescein isothiocyanate-labeled rabbit antibody to mouse immunoglobulins (Dakopatts, Copenhagen) for 30 min at 4°C. Washing was repeated in the same manner, and cell surface immunofluorescence was analyzed with an EPICS flow cytometer (Coulter Electronics, Hialeah, FL).

Deglycosylation of transfectants with benzyl-α-GalNAc Transfectants were incubated in 10% FCS/RPMI1640 supplemented with 5 m*M* benzyl-α-acetylgalactosamine (benzyl-α-GalNAc) (Sigma, St. Louis, MO) for 5 days. Benzyl-α-GalNAc is a competitive inhibitor of mucin glycosylation.[13] The cells were washed with RPMI1640, and flow cytometry was performed as described above to examine the effect of deglycosylation on the reactivity of transfectants with anti-MUC1 core protein mAb.

MTT assay To quantify the proliferation of the transfectants, we used colorimetric assay with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (Sigma)], as described by Ikeda *et al.*[14] Triplicate aliquots of 1×10^5 transfectant cells suspended in 100 ml of D-MEM containing 10% FCS were cultured in 96-well flat-bottomed microtiter plates for 48 h at 37°C. For the MTT assay, MTT (10 ml of a 5 mg/ml solution of MTT in PBS) was added to all wells, and plates were incubated for 4 h. Acid isopropanol (100 ml of 0.04 *N* HCl in isopropanol) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. The absorbance was then measured on a Microelisa plate reader (Dynatech Laboratories Inc., Alexandria, VA) at a wavelength of 570 nm.

Mitogenic response of transfectants to growth factors or extracellular matrices Incorporation of [^{3}H]thymidine into trichloroacetate-insoluble fraction was measured basically as described by Cai *et al.*[15] Triplicate aliquots of the 1×10^5 EJNIH3T3 transfectants in 1 ml of D-MEM supplemented with 0.2% FCS were seeded in 12-well culture plates. The cells were incubated at 37°C for 48 h to induce quiescence, then growth-stimulating factors were added. [^{3}H]Deoxythymidine (New England Nuclear, Wilmington, DE) was pulsed at the concentration of 1 mCi/ml at 20 h after stimulation. After incubation for 3 h, the cells were washed with cold PBS 3 times and fixed with 95% methanol. They were then washed with 10% trichloroacetate once and 5% trichloroacetate twice, lysed with 0.5 *N* NaOH for 10 min on ice, and neutralized with 1 *N* HCl. Finally, 10% (wt/vol) trichloroacetate was added. The solution (200 μl) was passed through a Whatman glass fiber filter and the radioactivity was measured with a liquid scintillation counter. Results are given as relative [^{3}H]thymidine incorporation, which is the ratio of [^{3}H]thymidine incorporation of transfectants cultured with growth factors to that without growth factors.

To test the mitogenic response to extracellular matrices, triplicate aliquots of the 1×10^5 EJNIH3T3 transfectants in 1 ml of D-MEM supplemented with 0.2% FCS were seeded in 35 mm collagen- or fibronectin-coated dishes (Corning, Corning, NY), and the same procedure as described above was employed.

Cell growth assay on extracellular matrices Triplicate aliquots of 1 ml of 1×10^5 EJNIH3T3 transfectants were seeded in 35 mm collagen- or fibronectin-coated dishes for 72 h. Non-treated dishes were used as a negative control. The number of cells was counted at 72 h using a hemocytometer.

Statistical analysis The significance of differences was determined by using Student's two tailed *t* test and Wilcoxon's rank test.

RESULTS

Inhibition of cell aggregation with MUC1 A previous study by Ligtenberg *et al.* demonstrated that SV40-immortalized normal human mammary epithelial HBL-100 cells and a human melanoma cell line A375, when transfected with MUC1 cDNA, did not aggregate as efficiently as their control cells.[8] An aggregation assay was performed to test whether the transfectants we established behave in the same manner (Fig. 1). MUC1$^-$ EJNIH3T3

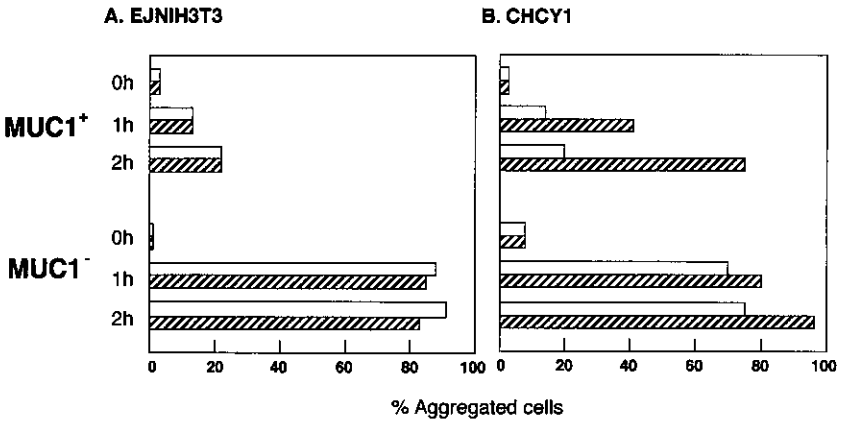

Fig. 1. Effect of MUC1 on the aggregation of EJNIH3T3 (A) and CHCY1 (B) cells. $MUC1^+$ and $MUC1^-$ indicate MUC1 transfectant and mock transfectant, respectively. Aggregation assays were performed under culture conditions with either no Ca^{2+} (open columns) or 10 m*M* Ca^{2+} (hatched columns). The ratio of number of aggregated cells to number of total cells is indicated as % aggregated cells.

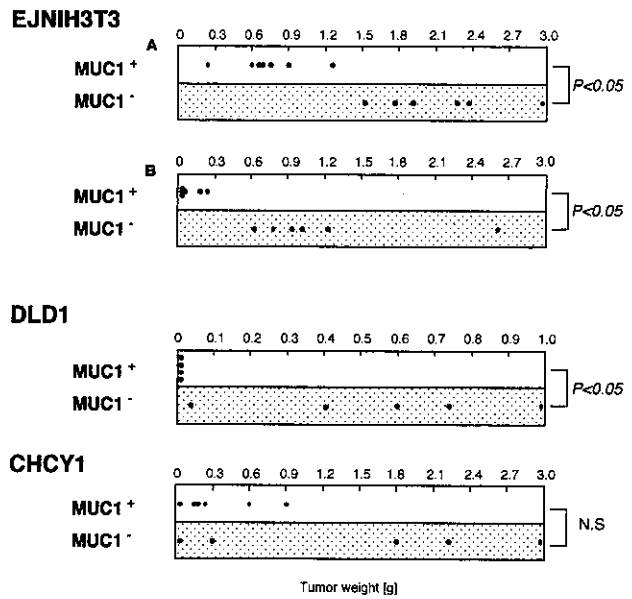

Fig. 2. Effect of MUC1 on *in vivo* growth of EJNIH3T3, DLD1 and CHCY1 cells. The weight (g) of the primary tumor was measured at 8 weeks after s.c. inoculation of transfectants. $MUC1^+$ and $MUC1^-$ indicate MUC1 transfectant and mock transfectant, respectively.

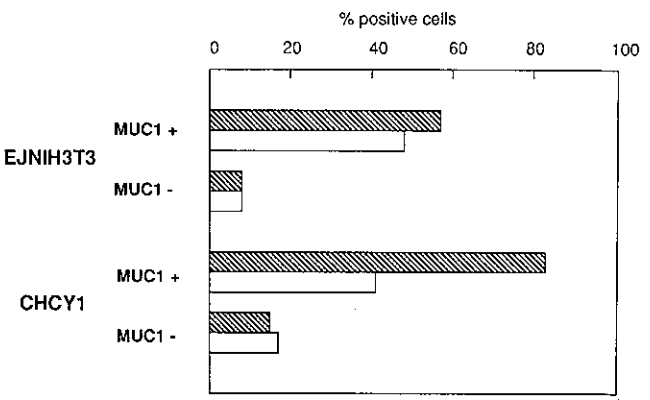

Fig. 3. Effect of benzyl-α-GalNAc on the reactivity of anti-MUC1 core protein mAb MUSE11 with MUC1 transfectants. $MUC1^+$ and $MUC1^-$ indicate MUC1 transfectant and mock transfectant, respectively. The reactivity of mAb was evaluated by flow cytometry. Benzyl-α-GalNAc: ▨, 5 m*M*; □, 0 m*M*.

cells (mock transfectant) aggregated strongly, i.e., over 80% of the cells showed aggregation at 1 h and 2 h after incubation, irrespective of the presence of Ca^{2+}. In contrast, only 15 to 20% of $MUC1^+$ EJNIH3T3 cells (MUC1 transfectant) aggregated, indicating the inhibitory activity of MUC1 on cell aggregation (Fig. 1A). In the case of CHCY1 cells too, the percentage of aggregated $MUC1^+$ CHCY1 cells was lower than that of $MUC1^-$ CHCY1 cells. Interestingly, Ca^{2+}-dependent cell aggregation was only partially inhibited by MUC1, suggesting the expression of some potent Ca^{2+}-dependent cell adhesion molecules on CHCY1 cells (Fig. 1B).

Effect of MUC1 on *in vivo* tumor cell growth In order to examine the biological significance of the potent anti-adhesion activity of MUC1 mucin, we observed the effect of MUC1 on *in vivo* tumor cell growth by subcutaneously inoculating $MUC1^+$ and $MUC1^-$ EJNIH3T3, CHCY1 and DLD1 cells into SCID mice. The primary tumor was weighed at 8 weeks after inoculation (Fig. 2). The tumor tissues of MUC1 transfectomas were positively stained by mAb MUSE11. The growth rate of $MUC1^+$ EJNIH3T3 cells was clearly lower than that of $MUC1^-$ EJNIH3T3 cells in the experiments with either 2×10^5 (A) or 2×10^6 (B) inoculated cells. The same tendency was observed in the case of $MUC1^+$ and $MUC1^-$

CHCY1 and DLD1 cells, where 2.0×10^6 cells were inoculated, although the difference of tumor weight between MUC1$^+$ and MUC1$^-$ cells was statistically significant in EJNIH3T3 and DLD1, but not in CHCY1. These results indicate that expression of MUC1 mucin suppresses the growth of these tumor cells.

Effect of benzyl-α-GalNAc on the reactivity of anti-MUC1 core protein mAb with transfectants The effect of benzyl-α-GalNAc on the reactivity of an anti-MUC1 core protein, mAb MUSE11, with transfectants was observed by flow cytometry to examine the influence of carbohydrate side chains on the access of the mAb to the protein core epitope. As shown in Fig. 3, the reactivity of mAb MUSE11 was not affected by benzyl-α-GalNAc in MUC1$^+$ EJNIH3T3 cells, whereas it was remarkably increased in the case of MUC1$^+$ CHCY1 cells. This suggests that the *O*-glycosylation of MUC1 protein on MUC1$^+$ EJNIH3T3 cells could be immature, indicating that these cells may be appropriate for analysis of the biological functions of MUC1 gene product.

Effect of MUC1 on *in vitro* tumor cell growth To clarify the mechanisms of the decreased *in vivo* tumor growth, the following *in vitro* experiments were performed using the EJNIH3T3 transfectant.

It was demonstrated by MTT assay that MUC1$^+$ EJNIH3T3 cells grew more slowly than MUC1$^-$ EJNIH3T3 cells in the exponential growing phase (Table I); their doubling times were 23.5 and 18.3 h, respectively. Thus, MUC1 had a negative effect on tumor cell growth *in vitro*.

Mitogenic response of EJNIH3T3 transfectants to growth factor or extracellular matrix The mitogenic response of MUC1$^+$ EJNIH3T3 cells to fetal calf serum or bombesin, a potent growth factor for fibroblasts,[16] was compared with that of MUC1$^-$ cells. As shown in Fig. 4, the DNA synthesis of MUC1$^+$ EJNIH3T3 cells after stimulation with either FCS or bombesin was significantly lower than that of MUC1$^-$ cells. Significant reduction of growth stimulation of EJNIH3T3 transfectants by extracellular matrix, collagen or fibronectin, was also observed (Fig. 5). These data suggest that MUC1 inhibits both growth factor-to-cell surface receptor and extracellular matrix-to-cell surface receptor interactions.

Effect of MUC1 on the growth of EJNIH3T3 cells on extracellular matrix The growth rate of EJNIH3T3 transfectants on a collagen- or fibronectin-coated dish was measured by counting the cell number at 72 h after

Table I. Effect of MUC1 on *in vitro* Tumor Cell Growth

	Transfectants	Absorbance[a]	Number of cells	
Day 0	MUC1$^+$	0.094±0.009	5740±310	NS
	MUC1$^-$	0.093±0.002	5710±70	
Day 3	MUC1$^+$	0.450±0.018	50410±5850	$P<0.05$
	MUC1$^-$	0.540±0.015	87300±8300	
Day 6	MUC1$^+$	0.599±0.049	125100±43610	NS
	MUC1$^-$	0.576±0.058	108750±46170	

Doubling time: MUC1 transfectant 23.5 h, mock transfectant 18.3 h.
a) MTT assay.

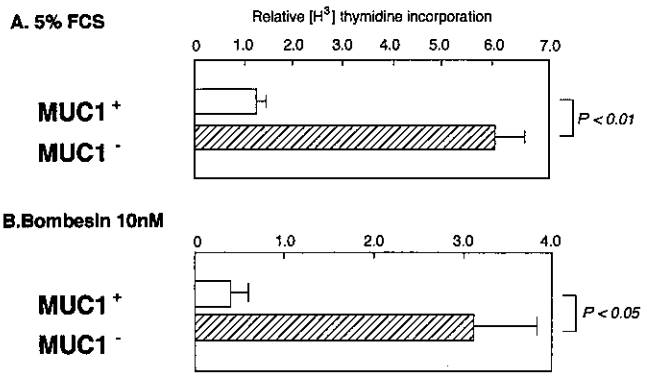

Fig. 4. Effect of MUC1 on the mitogenic response of EJNIH3T3 cells to 5% FCS (A) or 10 n*M* bombesin (B). The relative [^{3}H]thymidine incorporation is the ratio of [^{3}H]-thymidine incorporation of transfectants cultured with growth factors to that without growth factors. MUC1$^+$ (open columns) and MUC1$^-$ (hatched columns) indicate MUC1 transfectant and mock transfectant, respectively. Values are shown as mean±SD of triplicate determinations.

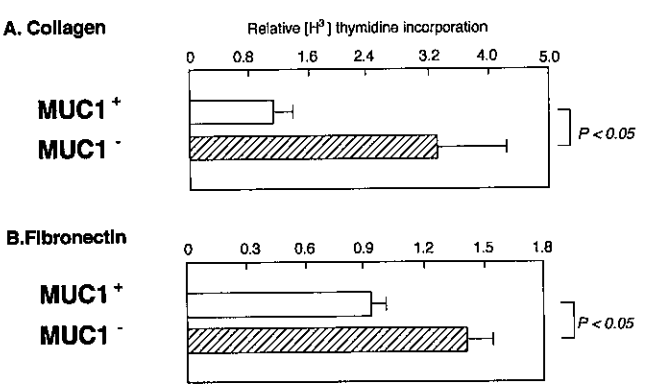

Fig. 5. Effect of MUC1 on the mitogenic response of EJNIH3T3 cells to collagen (A) or fibronectin (B). The relative [^{3}H]thymidine incorporation is the ratio of [^{3}H]thymidine incorporation of transfectants cultured in the dish coated with extracellular matrix to that without extracellular matrix. The MUC1$^+$ (open columns) and MUC1$^-$ (hatched columns) indicate MUC1 transfectant and mock transfectant, respectively. Values are shown as mean±SD of triplicate deteminations.

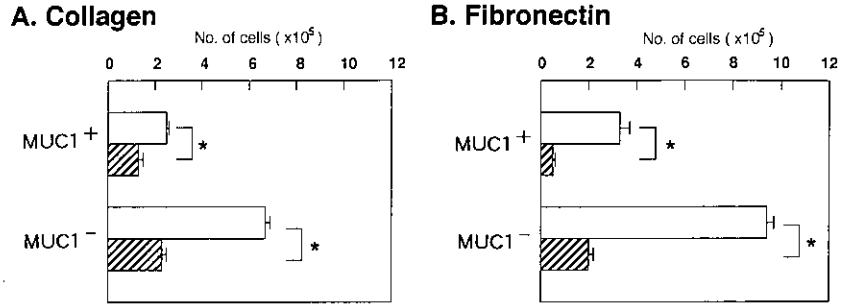

Fig. 6. Effect of MUC1 on the growth of EJNIH3T3 transfectant in collagen (A)- or fibronectin (B)-coated dishes (open columns) or in non-treated dishes (hatched columns). The number of cells at 72 h after incubation was counted. MUC1$^+$ and MUC1$^-$ indicate MUC1 transfectant and mock transfectant, respectively. Values are shown as mean±SD of triplicate determinations. □, collagen- or fibronectin-coated dish; ▨, non treated dish. * $P<0.01$.

incubation (Fig. 6). As anticipated from the results in Fig. 5, MUC1$^+$ EJNIH3T3 cells grew more slowly on both collagen- and fibronectin-coated dishes than MUC1$^-$ cells did. It should be noted here that the growth of EJNIH3T3 cells was consistently poor, independent of the expression of MUC1, when cultured in non-treated dishes, indicating that interaction with extracellular matrix could be essential to the growth of EJNIH3T3 cells.

DISCUSSION

We transfected MUC1 cDNA into an H-*ras*-transformed mouse fibroblast cell line, EJNIH3T3, and a human colon cancer cell line, CHCY1, resulting in a remarkable decrease of the aggregation activity of both cell lines. This supports the idea that MUC1 functions as an anti-adhesion molecule.[8,9] The inhibition of cell-cell interaction by MUC1 in tumor cells appears to be equivalent to the loss of adhesion molecules, which has been shown to affect profoundly various biological properties of tumor cells, including growth rate, differentiation level, invasion and metastasis.[17–19] We therefore examined the effect of MUC1 on tumor cell growth in SCID mice, and found that MUC1 overexpression clearly lowered the growth rate of EJNIH3T3 cells and human colon cancer DLD1 cells. SCID mice were used to exclude the possibility of an immune response to human MUC1 as a reason for decreased tumor growth.

To elucidate the mechanism for the decreased growth rate of tumor cells upon expression of MUC1, several *in vitro* experiments were performed using EJNIH3T3 transfectants. First, cell growth was observed by MTT assay. MUC1$^+$ EJNIH3T3 cells grew more slowly than MUC1$^-$ EJNIH3T3 cells in the exponential growth phase, suggesting that the decreased growth of the primary tumor of MUC1$^+$ EJNIH3T3 cells in SCID mice is partly due to decreased proliferative activity of the cells themselves. Secondly, the effect of MUC1 on the mitogenic response of transfectants to growth factors was observed. Bombesin is known to stimulate cell growth through the rapid tyrosine phosphorylation of focal adhesion kinase (p125^{FAK}) as well as other neuropeptides in Swiss 3T3 cells.[20] The DNA synthesis of MUC1$^+$ EJNIH3T3 cells after stimulation with either FCS or bombesin was much lower than that of MUC1$^-$ cells. Although the precise mechanism of the inhibition of growth factor-receptor interaction by MUC1 is not clear at present, one possible explanation is the binding of growth factors to MUC1. It has been shown that proteoglycans, proteins with large carbohydrate chains composed of repeating disaccharide units, function as modulators of growth factors.[21] Growth factors that bind to heparin and heparan sulfate include fibroblast growth factors, granulocyte-macrophage colony stimulating factor, interleukin-3 and platelet factor 4.[22–24] Moreover, transforming growth factor β binds to proteoglycans through the core protein.[25] Alternatively, growth factor receptors may be down-regulated by the overexpression of MUC1. When a pancreas cancer cell line with poorly differentiated phenotype was transfected with MUC1 cDNA, some differentiated phenotypes appeared,[26] suggesting the induction of cell differentiation by the expression of MUC1.

Thirdly, the growth rate of MUC1$^+$ EJNIH3T3 cells on collagen- or fibronectin-coated dishes was remarkably reduced compared to that of MUC1$^-$ cells. A possible mechanism for the inhibition of cell-matrix interaction with MUC1 is steric hindrance, as has been observed in the inhibition of cell aggregation. Proteoglycans have been shown to interfere with cell-matrix adhesion, probably by steric hindrance, due to their large and highly charged structures.[27] In addition, a glycoprotein, tenascin, inhibits integrin-mediated chick fibroblast attachment to fibronectin, laminin, and the GRGDS peptide.[28] Cell differentiation induced by the overexpression of MUC1 is implausible as a cause of lowered expression of integrins; it has been demonstrated that loss of an integrin, VLA5, could be related to transformation of human colonic epithelial cells,[29] and gives rise to increased tumorigenicity of a human colon cancer cell line in nude mice.[19] The matrix-integrin interaction stimu-

lates tyrosine phosphorylation of p125[FAK 30)] and MAPK activation,[31)] and these alterations of cell signaling mechanisms have important effects on cell growth and cell differentiation. MUC1 mucin may have profound effects on this signal transduction pathway.

Since MUC1 mucin is expressed with high frequency in a variety of tumors,[7)] it is conceivable that MUC1 may be an advantageous phenotype for *in vivo* tumor development. The data presented in this paper suggest that MUC1 could negatively regulate the growth of tumor cells through interference with cell-cell, growth factor-receptor and cell-matrix interactions, eventually giving them an appropriate growth rate and other properties favorable for self-protection,[32)] invasion and metastasis.[33, 34)]

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REFERENCES

1) Lightenberg, M. J. L., Vos, H. L., Gennissen, A. M. C. and Hilkens, J. Episialin, a carcinoma-associated mucin, is generated by a polymorphic gene encoding splice variants with alternative amino termini. *J. Biol. Chem.*, **265**, 5573–5578 (1990).
2) Wreshner, D. H., Hareuveni, M., Tsarfaty, I., Smorodinsky, N., Horev, J., Zaretsky, J., Kotkes, P., Weiss, M., Lathe, R., Dion, A. and Keydar, I. Human epithelial tumor antigen cDNA sequences. Differential splicing may generate multiple protein forms. *Eur. J. Biochem.*, **189**, 463–473 (1990).
3) Gendler, S. J., Lancaster, C. A., Taylor-Papadimitriou, J., Duhig, T., Peat, N., Burchell, J., Lalani, E.-N. and Wilson, D. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J. Biol. Chem.*, **265**, 15286–15293 (1990).
4) Lan, M. S., Batra, S. K., Qui, W.-N., Metzger, R. S. and Hollingsworth, M. A. Cloning and sequencing of a human pancreatic mucin cDNA. *J. Biol. Chem.*, **265**, 15294–15299 (1990).
5) Gum, J. R. Mucin genes and the proteins they encode: structure, diversity, and regulation. *Am. J. Respr. Cell. Mol. Biol.*, **7**, 557–564 (1992).
6) Nishimori, I., Perini, F., Mountjoy, K. P., Sanderson, S. D., Johnson, N., Cerny, R. L., Gross, M. L., Darell Fontenot, J. and Hollingsworth, M. A. N-Acetylgalactosamine glycosylation of MUC1 tandem repeat peptides by pancreatic tumor cell extracts. *Cancer Res.*, **54**, 3738–3744 (1994).
7) Ho, S. B., Niehaus, G. A., Lyftogt, C., Yan, P. S., Cherwitz, D. L., Gum, E. T., Dahiya, R. and Kim, Y. S. Heterogeneity of mucin gene expression in normal and neoplastic tissues. *Cancer Res.*, **53**, 641–651 (1993).
8) Lightenberg, M. J. L., Buijs, F., Vos, H. L. and Hilkens, J. Suppression of cellular aggregation by high level of episialin. *Cancer Res.*, **52**, 2318–2324 (1992).
9) van de Wiel-van Kemenade, E., Lightenberg, M. J. L., de Boer, A. J., Buijs, F., Vos, H. L., Melief, C. J. M., Hilkens, J. and Figdor, C. G. Episialin (MUC1) inhibits cytotoxic lymphocyte-target cell interaction. *J. Immunol.*, **151**, 767–776 (1993).
10) Ban, T., Imai, K. and Yachi, A. Immunohistological and immunochemical characterization of a novel pancreatic cancer-associated antigen MUSE11. *Cancer Res.*, **49**, 7141–7146 (1989).
11) Hinoda, Y., Nakagawa, N., Ohe, Y., Kakiuchi, H., Tsujisaki, M., Imai, K. and Yachi, A. Recognition of the polypeptide core of mucin by monoclonal antibody MUSE11 against an adenocarcinoma-associated antigen. *Jpn. J. Cancer Res.*, **81**, 1206–1209 (1990).
12) Rojas, M., Fuks, A. and Stanners, C. P. Biliary glycoprotein, a member of the immunoglobulin supergene family, functions *in vitro* as a Ca^{2+}-dependent intercellular adhesion molecule. *Cell Growth Differ.*, **1**, 527–533 (1990).
13) Kuan, S. F., Byrd, J. C., Basbaum, C. B. and Kim, Y. S. Inhibition of mucin glycosylation by aryl-N-acetyl-α-galactosaminides in human colon cancer cells. *J. Biol. Chem.*, **264**, 19271–19277 (1989).
14) Ikeda, H., Kanakura, Y., Takami, T., Kuriu, A., Kitayama, H., Ishikawa, J., Kanayama, Y., Yonezawa, T., Tarui, S. and Griffin, J. D. Expression and functional role of the proto-oncogene c-*kit* in acute myeloblastic leukemia for stem cell factor. *Blood*, **78**, 2962–2968 (1991).
15) Cai, H., Szeberenyi, J. and Cooper, G. M. Effect of a dominant inhibitory Ha-*ras* mutation on mitogenic signal transduction in NIH3T3 cells. *Mol. Cell. Biol.*, **10**, 5314–5323 (1990).
16) Woll, P. J. and Rozengurt, E. Bombesin and bombesin antagonists: studies in Swiss 3T3 cells and human small cell lung cancer. *Br. J. Cancer*, **57**, 579–586 (1988).
17) Vleminckx, K., Vakaet, L., Jr., Mareel, M., Fiers, W. and van Roy, F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell*, **66**, 107–119 (1991).
18) Schipper, J. H., Frixen, U. H., Behrens, J., Unger, A., Jahnke, K. and Birchmeier, W. E-cadherin expression in squamous cell carcinomas of head and neck: inverse correlation with tumor dedifferentiation and lymph node metas-

tasis. *Cancer Res.*, **51,** 6328–6337 (1991).
19) Stallmach, A., Lampe, B. V., Orzechowski, H.-D., Matthes, H. and Ricken, E.-O. Increased fibronectin-receptor expression in colon carcinoma-derived HT29 cells decreases tumorigenicity in nude mice. *Gastroenterology,* **106,** 19–27 (1994).
20) Zachary, I., Sinnett-Smith, J. and Rozengurt, E. Bombesin, vasopressin, and endothelin stimulation of tyrosine phosphorylation in Swiss 3T3 cells. *J. Biol. Chem.*, **267,** 19031–19034 (1992).
21) Ruoslahti, E. and Yamaguchi, Y. Proteoglycans as modulators of growth factor activities. *Cell,* **64,** 867–869 (1991).
22) Yayon, A., Klagsbrun, M., Esko, J. D., Leder, P. and Ornitz, D. M. Cell surface, heparin-like molecules are required for binding of fibroblast growth factor to its high affinity receptor. *Cell,* **64,** 841–848 (1991).
23) Roberts, R., Gallagher, J., Spooncer, E., Allen, T. D., Bloomfield, F. and Dexter, T. M. Heparan sulfate bound growth factors: a mechanism for stromal cell mediated hematopoiesis. *Nature,* **332,** 376–378 (1988).
24) Li, Y.-S., Milner, P. G., Chauhan, A. K., Watson, M. A., Hoffman, R. M., Kodner, C. M., Milbrandt, J. and Deuel, T. F. Cloning and expression of a developmentally regulated protein that induces mitogenic and neurite outgrowth activity. *Science,* **250,** 1690–1694 (1990).
25) Yamaguchi, Y., Mann, D. M. and Ruoslahti, E. Negative regulation of transforming growth factor-β by the proteoglycan decorin. *Nature,* **346,** 281–284 (1990).
26) Batra, S. K., Lern, H. F., Worlock, A. J., Metzgar, R. S. and Hollingsworth, M. A. Transfection of the human MUC1 mucin gene into a poorly differentiated human pancreatic tumor cell line, Panc1: integration, expression and ultrastructural changes. *J. Cell Sci.,* **100,** 841–849 (1991).
27) Yamagata, M., Suzuki, S., Akiyama, S. K., Yamada, K. M. and Kimata, K. Regulation of cell-substrate adhesion by proteoglycans immobilized on extracellular substances. *J. Biol. Chem.*, **264,** 8012–8018 (1989).
28) Chiquet-Ehrismann, R., Kalla, P., Person, C. A., Beck, K. and Chiquet, M. Tenascin interferes with fibronectin action. *Cell,* **53,** 383–390 (1988).
29) Stallmach, A., Lampe, B. V., Mattehes, G., Bornhoft, G. and Riecken, E. O. Differential loss of integrin adhesion molecules on human colonic epithelial cells during the benign to malignant tumor transformation. *Gut,* **33,** 342–346 (1992).
30) Guan, J. and Shalloway, D. Regulation of focal adhesion associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation. *Nature,* **358,** 690–692 (1992).
31) Chen, Q., Kinch, M. S., Lin, T. H., Burridge, K. and Juliano, R. L. Integrin mediated cell adhesion activates mitogen-activated protein kinases. *J. Biol. Chem.,* **269,** 26602–26605 (1994).
32) Jentoft, N. Why are proteins O-glycosylated? *Trends Biochem. Sci.,* **15,** 291–294 (1990).
33) Hilkens, J., Lightenberg, M. J. L., Vos, H. L. and Litvinov, S. V. Cell membrane-associated mucins and their adhesion-modulating property. *Trends Biochem. Sci.,* **17,** 359–363 (1992).
34) Nakamori, S., Ota, D. M., Cleary, K. R., Shirotani, K. and Irimura, T. MUC1 mucin expression as a marker of progression and metastasis of human colorectal carcinoma. *Gastroenterology,* **106,** 353–361 (1994).