The enzyme GnT-III (β1,4-N-acetylglucosaminyltransferase III) catalyzes the addition of a bisecting N-acetylglucosamine (GlcNAc) residue on glycoproteins. Our previous study described that the transfection of GnT-III into mouse melanoma cells results in the enhanced expression of E-cadherin, which in turn leads to the suppression of lung metastasis. It has recently been proposed that the phosphorylation of a tyrosine residue of β-catenin is associated with cell migration. The present study reports on the importance of bisecting GlcNAc residues by GnT-III on tyrosine phosphorylation of β-catenin using three types of cancer cell lines. An addition of bisecting GlcNAc residues to E-cadherin leads to an alteration in cell morphology and the localization of β-catenin after epidermal growth factor stimulation. These changes are the result of a down-regulation in the tyrosine phosphorylation of β-catenin. In addition, tyrosine phosphorylation of β-catenin by transfection of constitutively active c-src was suppressed in GnT-III transfectants as well as in the case of epidermal growth factor stimulation. Treatment with tunicamycin abolished any differences in β-catenin phosphorylation for the mock vis à vis the GnT-III transfectants. Thus, the addition of a specific N-glycan structure, the bisecting GlcNAc to E-cadherin-β-catenin complex, down-regulates the intracellular signaling pathway, suggesting its implication in cell motility and the suppression of cancer metastasis.

The remodeling of cell surface oligosaccharides by glycosyltransferase gene transfection has led to a better understanding of the biological functions of several glycoproteins (reviewed in Ref. 1). Changes in adhesion molecules and growth factor receptors by modification of their oligosaccharide structure are associated with the function and the biological behavior of cancer cells (2–4). These phenomena appear to be linked to the progression of cancer metastasis and invasion. However, the mechanism for this is complicated. One of the likely contributing molecules is the homophilic adhesion molecule, E-cadherin (5, 6), a decreased expression of which is highly associated with the initial step of metastasis in certain types of cancer (7–10). E-cadherin contains five consensus N-glycosylation sites. These N-glycans on E-cadherin can be modified with a bisecting N-acetylglucosamine (GlcNAc) structure, which is the product of β1,4-N-acetylglucosaminyltransferase III (GnT-III, EC 2.4.1.144) (11). GnT-III catalyzes the attachment of a GlcNAc residue to a β1–4 mannose residue in the core region of N-glycans, as shown in Fig. 1.

In an earlier study, we demonstrated that the bisecting GlcNAc on E-cadherin suppressed lung metastasis of melanoma cells (12). However, the mechanism has not been fully investigated. The function of E-cadherin is also controlled via molecules from the cytoplasm, namely α-, β-, and γ-catenins (13, 14). The deletion or the mutational inactivation of β-catenin leads to the abolition of E-cadherin activity and tumor invasion (15, 16). Recently, the biological significance of the tyrosine phosphorylation of β-catenin has been examined by several groups (17–20). It is possible that this phosphorylation could disturb E-cadherin/cytoskeleton interactions (17). During cell migration, β-catenin accumulates in the cytosol in a free, uncomplexed, and tyrosine-phosphorylated form (19). In contrast, this type of β-catenin is not observed during the quiescent phase, suggesting that the tyrosine phosphorylation of β-catenin is linked to cell migration (20). Thus, increases in tyrosine-phosphorylated β-catenin are thought to contribute to the malignant progression and metastasis of tumor cells.

It has been reported that EGFr or v-src phosphorylates β-catenin in the case of breast cancer and MDCK cells (21–23). EGFr receptor (EGF-R) is also a glycoprotein, and its sugar chain represents a potential substrate for GnT-III as well as E-cadherin. Therefore, the tyrosine phosphorylation of β-catenin could be influenced by both aberrantly glycosylated EGF-R and E-cadherin. In this study, we report on an investigation of the effects of oligosaccharide modification by GnT-III on the tyrosine phosphorylation of β-catenin and cell mobility using

---

* This work was supported in part by a Grant-in-aid for Scientific Research on Priority Areas 10178104 from the Ministry of Education, Science, Sports, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†† To whom correspondence should be addressed. Tel.: 81-6-6879-3420; Fax: 81-6-6879-3429; E-mail: proftan@biochem.med.osaka-u.ac.jp.

---

The JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 276, No. 1, Issue of January 5, pp. 475–480, 2001

Printed in U.S.A. © 2001 by The American Society for Biochemistry and Molecular Biology, Inc.
β-Catenin and GnT-III

Three types of cancer cell lines. Our findings show that the pattern of β-catenin tyrosine phosphorylation as the result of stimulation by EGF or transfection of constitutively active c-src was dramatically changed in the case of GnT-III transfectants. Possible mechanisms for this process are discussed.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dulbecco's modified Eagle's medium and RPMI were obtained from Nihon Biomedical Laboratory (Kyoto, Japan). Penicillin was obtained from Banyu Corp. (Tokyo, Japan). Kanamycin, G418, and tunicamycin were obtained from Sigma. LipofectAmine reagent was obtained from Life Technologies, Inc. Effectene transfection reagent was obtained from Qiagen. Biotinylated erythroagglutinating phytohemagglutinin (E4-PHA) lectin was obtained from Ronen Corp. (Tokyo, Japan). A monoclonal antibody against human β-catenin was obtained from Chemicon International Inc. (Temecula, CA). A sheep polyclonal antibody against human EGF-R was obtained from Upstate Biotechnology (Lake Placid, NY). A mouse monoclonal antibody against human GnT-III was obtained from Fujirebio (Hachioji, Japan). A monoclonal antibody against E-cadherin and a monoclonal antibody against phosphotyrosine (PY20) were obtained from Transduction Laboratories (Lexington, KY). A peroxidase-conjugated goat antibody against mouse IgG was obtained from Promega (Madison, WI). A peroxidase-conjugated goat antibody against sheep IgG was obtained from Dako (Kyoto, Japan). A detection kit for horseradish peroxidase-avidin complex (ABC kit) was obtained from Vector Laboratories (Burlingame, CA). Fluorescein isothiocyanate-labeled goat antibody against mouse IgG was obtained from Dickinson Immunocytometry Systems (San Jose, CA). EGF was obtained from Wakunaga (Hiroshima, Japan). Polyvinylidene difluoride membrane was obtained from Millipore Corp. (Bedford, MA). A Zeta-probe membrane was obtained from Bio-Rad. Protein G-Sepharose 4 FF beads and enhanced chemiluminescence system (ECL) were obtained from Amersham Pharmacia Biotech. Permaflour aqueous mounting medium was obtained from Immunon. The biocinchonic acid (BCA) Kit was obtained from Pierce.

**Establishment of GnT-III-transfected B16-hm, Huh7, and WiDr Cells**—A mouse melanoma cell line, B16-hm was established from B16-F1 cells as described previously (12). A human colon cancer cell line, WiDr, was provided by the American Type Culture Collection (Manassas, VA). A human hepatoma cell line, Huh7, was provided by the Japanese Health Science Foundation (Tokyo, Japan). B16-hm cells were cultured in Dulbecco's modified Eagle's medium, and Huh7 and WiDr cells were cultured in RPMI. Each medium contained 100 μg/ml kanamycin, 50 units/ml penicillin, and 10% fetal calf serum. Cell lines were plated using the method reported previously (27). Approximately 20 μg of RNAs were electrophoresed on a 1% agarose gel containing 2.2 μM formamide and transferred onto a Zeta-probe membrane by capillary action. Northern blot analysis of GnT-III was performed as reported previously (28).

**Western Blot Analysis**—Twenty micrograms of proteins that had been extracted from mock and GnT-III transfectants of B16-hm, Huh7, and WiDr cells were electrophoresed on a 5% polyacrylamide gel and then transferred onto a polyvinylidene difluoride membrane. After blocking with phosphate-buffered saline (PBS) containing 3% bovine serum albumin overnight at 4 °C, the membrane filter was incubated with anti-human E-cadherin and anti-EGF-R and diluted with Tris-buffered saline containing 0.5% Tween 20 (TBS-T, 1:1000) for 2 h at room temperature. Each filter was washed 3 times with TBS-T for 10 min each and then incubated with TBS-T containing peroxidase-conjugated goat antibody to mouse IgG for anti-E-cadherin and rabbit antibody to sheep IgG for anti-EGF-R diluted to 1:2500 with TBS-T for 1 h at room temperature. After the membrane was washed 3 times with TBS-T for 10 min each, it was developed by an ECL according to the manufacturer's recommended protocol.

**Lectin Blot Analysis of Immunoprecipitated E-cadherin**—Mock and GnT-III transfectants of WiDr cells were plated on 10-cm dishes and incubated for 72 h. After washing with PBS, cells were lysed in a lysis buffer (10 mM Tris-HCl, pH 7.8, 0.15 M NaCl, 1 mM EDTA, 0.5% SDS, 0.5% Triton X-100, 2 mM CaCl₂, 10% (v/v) glycerol, 1 mM phenylmethanesulfonyl fluoride, 1 mM pepstatin, 10 μg/ml leupepton, and 10 μg/ml aprotonin) for 30 min on ice and precleared by centrifugation at 15000 rpm for 15 min at 4 °C. For immunoprecipitation, 15 μl of protein G-Sepharose 4FF beads were added to the above supernatant and rotator for 1 h at 4 °C with end-over-end rotations. Immunocomplexes were collected with 15 μl of protein G-Sepharose 4 FF beads. After washing with a lysis buffer 5 times, the complexes were released by boiling in Laemmli sample buffer, separated by 6% SDS-polyacrylamide gel electrophoresis, and electrotransferred onto a polyvinylidene difluoride membrane. The membrane was blocked in PBS containing 5% bovine serum albumin and incubated for 1 h with E4-PHA lectin (10 μg/ml). E4-PHA lectin binds preferentially to bisecting GlcNAc residues, and the binding of E4-PHA is enhanced by the presence of bisecting GlcNAc structures (29). After washing 3 times with TBS-T for 10 min each, the membrane was incubated with TBS-T containing ABC kit for 1 h. After the membrane was washed 3 times with TBS-T for 10 min each, it was developed by ECL according to the manufacturer's protocol. After submerging the membrane in 10% methanol, Tris-HCl (100 mM, pH 7.5), Tris-Cl (100 mM, pH 8.0), and 2-mercaptoethanol) at 50 °C for 1 h, the membrane was blocked with PBS containing 3% bovine serum albumin at 4 °C overnight, and then it was subjected to Western blot analysis using an anti-E-cadherin monoclonal antibody as described above.

**Cell Migration and Immunofluorescence**—Mock and GnT-III transfectants of WiDr cells were plated at a density of 1 × 10⁵ cells/cm² on 6-cm dishes. The mobility of mock and GnT-III transfectants of WiDr cells after EGF treatment were continuously observed with an optical microscope Diaphoto 300 (Nikon, Japan). The intracellular distribution of β-catenin in mock and GnT-III transfectants of WiDr cells was investigated by photofluorescent immunohistochemical methods. Briefly, cells were plated at a density of 1 × 10⁶ cells/cm² on 8-well chamber slides, cultured for 48 h, and incubated for 24 h in RPMI or Dulbecco's modified Eagle's medium containing 2% fetal calf serum. After treatment with EGF at the indicated time intervals, cells were washed twice with PBS, fixed with 3% paraformaldehyde in PBS for 20 min at room temperature, blocked, and permeabilized with 1% saponin and 1% bovine serum albumin in PBS for 20 min at 4 °C. The cells were incubated with 1:50-diluted anti-β-catenin antibody at room temperature for 30 min at 4 °C with 1% bovine serum albumin. Primary antibody binding was detected with a fluorescein isothiocyanate-labeled goat antibody to mouse IgG for 1 h at room temperature. Coverslips were mounted under a Permaflour aqueous mounting medium. Stained cells were viewed with a laser scanning confocal microscope LSM 410 (Carl Zeiss, Germany) and subsequently handled using Adobe Photoshop.
Fig. 2. Expression of GnT-III mRNA and proteins. A, 20 μg of total RNAs extracted from mock and GnT-III-transfected WiDr cells were electrophoresed on a 1.0% agarose gel containing 2.2 mM formaldehyde and then analyzed by Northern blot hybridization using 32P-labeled GnT-III cDNA (upper panel). Ethidium staining indicates that comparable amounts of RNAs were present. The ribosomal RNA, as visualized by ethidium staining, indicate that equal amounts of RNAs were loaded in each lane (lower panel). B, approximately 20 μg of proteins extracted from mock and GnT-III transfectants of WiDr and Huh7 cells were subjected to an 8% SDS-polyacrylamide gel electrophoresis. Expression of GnT-III was analyzed by Western blotting using a specific antibody to GnT-III. Lane 1 indicates WiDr-M1, lane 2 indicates WiDr-M2, lane 3 indicates WiDr-GT1, lane 4 indicates WiDr-GT2, lane 5 indicates Huh7-M, and lane 6 indicates Huh7-GIII. ND, not detected.

Tyrosine Phosphorylation of β-Catenin and EGF-R—Mock and GnT-III transfectants of WiDr, B16-hm, and Huh7 cells were plated at a density of 4 × 10^4 cells/cm² on 10-cm dishes and incubated in normal conditions for 72 h. Those cells, in a subconfluent state, were further cultured for 24 h in RPMI or Dulbecco’s modified Eagle’s medium (Nikken, Kyoto, Japan) containing 2% fetal calf serum and were then treated with 50 ng/ml EGF. After washing with an ice-cold solution of PBS for the indicated time intervals, the cells were lysed in an ice-cold lysis buffer (5 mM EDTA, 1 mM sodium orthovanadate, 1 mM sodium pyrophosphate, 100 mM sodium fluoride, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin, 10 μM leupeptin) for 30 min on ice and precleared to remove pellets (insoluble fraction) by centrifugation at 15000 rpm for 15 min at 4 °C. Immunoprecipitation was performed using anti-β-catenin or anti-EGF-R antibody. Bound proteins were subjected to 6% SDS-polyacrylamide gel electrophoresis and detected by immunoblotting with anti-PY20. After reprobing and blocking the membrane, it was subjected to Western blot analysis using anti-β-catenin or EGF-R as described above.

Tunicamycin Treatment—To further investigate the importance of oligosaccharides on β-catenin phosphorylation, cells were treated with 0.5 μg/ml tunicamycin (Sigma) for 24 h before EGF stimulation.

Tyrosine Phosphorylation of β-Catenin by the Transfection of a Constitutively Active Form of c-src—The src expression vector, c-srcY527F, which is regulated by SV40 promoter and constitutively activated by mutation from 527 tyrosine to phenylalanine, was kindly provided by Dr. Fukushima (Ishida City Hospital, Osaka, Japan) (30). This vector was transfected into mock and GnT-III transfectants of WiDr cells with Effectene according to the standard protocol. After 48 h, the tyrosine phosphorylation of β-catenin in these cells was investigated as described above.

RESULTS

Establishment of GnT-III-transfected WiDr and Huh7 Cells—Although the GnT-III activity of WiDr and Huh7 cells was quite low, WiDr-GT1, -GT2, and Huh7-GIII showed >100 times higher levels of GnT-III activity than their mock transfectants (Fig. 2). Northern blot analysis showed a high level of expression of GnT-III mRNA in GnT-III-transfected WiDr and Huh7 cells, which is consistent with the observed enzyme activity (Fig. 2A) and suggests that the high levels of GnT-III activity in WiDr-GT1, -GT2, and Huh7-GIII were due to the high transcriptional levels. Western blot analysis showed a high level of GnT-III protein in GnT-III transfectants (Fig. 2B). Similar results of GnT-III expression were observed in GnT-III-transfected B16-hm cells (12).

Western Blot and Lectin Blot Analyses of E-cadherin and EGF-R—The expression of E-cadherin and EGF-R was investigated by Western blot analysis. Whereas the expression of E-cadherin in B16-hm cells was increased in the case of GnT-III transfectants compared with mock transfectants (3), the level in the GnT-III transfectants of WiDr and Huh7 cells was nearly the same compared with each mock transfectant. However, the molecular size of E-cadherin in GnT-III-transfected WiDr and Huh7 cells was smaller than that in the mock transfectants, suggesting that an extension of N-oligosaccharide in E-cadherin had been suppressed by the action of GnT-III (Fig. 3A, upper panel). The level of EGF-R expression was very low in both mock and GnT-III transfectants of B16-hm cells. In contrast, a high level of expression of EGF-R was observed in WiDr and Huh7 cells, and this level was nearly equal in both the mock and the GnT-III transfectants. The molecular size of EGF-R in GnT-III-transfected WiDr and Huh7 cells was smaller than that of the mock transfectants as well as in the case of E-cadherin (Fig. 3A, lower panel).

To further investigate the source of the differences in the molecular size of E-cadherin in mock and GnT-III transfectants, lectin blot analysis on immunoprecipitated E-cadherin was performed using biotinylated E4-PHA. E-cadherin, which had been immunoprecipitated from WiDr-GT1, -GT2, and Huh7-GIII cells exhibited an increase in E4-PHA binding compared with mock transfectants (Fig. 3B, a). An increase in E4-PHA
binding to EGF-R immunoprecipitated from GnT-III transfectants was also observed (Fig. 3B, b). These results indicate that GnT-III transfection led to an increase in the number of bisecting GlcNAc residues on the N-glycans of E-cadherin and EGF-R in WiDr and Huh7 cells.

**Cell Morphology and Distribution of β-Catenin after EGF Treatment**—When the cells were treated with EGF, the degree of cell-cell contacts became less strong in the mock cells. A typical pattern of changes in cell morphology was observed in WiDr cells (Fig. 4A). After 60 min, the morphology of the mock cells returned to the original shape. This change, however, was negligible in the case of GnT-III transfectants. It is interesting to note that the intracellular localization of β-catenin was altered along with the morphological alteration of mock transfectants (Fig. 4B). The localization of β-catenin was changed from the cell membrane to the cytoplasm after EGF treatment. In the GnT-III transfectants, most of the β-catenin continued to remain distributed along the entire cell membrane after EGF treatment.

**Tyrosine Phosphorylation of β-Catenin after EGF Treatment**—Since EGF treatment is a well known method for stimulating the tyrosine phosphorylation of β-catenin, GnT-III-transfected WiDr, B16-hm, and Huh7 cells were treated with 50 ng/ml EGF. As shown in Fig. 5, a dramatic difference in tyrosine phosphorylation of β-catenin was observed between GnT-III and mock transfectants. When EGF was added to mock-transfected WiDr cells, the tyrosine phosphorylation of β-catenin increased within 15 min of EGF stimulation and persisted for at least 60 min. In contrast, the levels of tyrosine phosphorylation of β-catenin were very low and increased only slightly at 15 min after EGF stimulation and then decreased in the GnT-III transfectants (Fig. 5A, upper panel). In the case of B16-hm cells, the basal level of tyrosine phosphorylation of β-catenin before EGF treatment was somewhat higher than that in the WiDr cells. The tyrosine phosphorylation of β-catenin was slightly increased in mock cells after EGF treatment but was dramatically decreased in GnT-III transfectants (Fig. 5B, upper panel). In the case of Huh7 cells, the tyrosine phosphorylation of β-catenin was much weaker than the B16-hm and WiDr cells. However, the difference of β-catenin phospho-
Tyrosine phosphorylation of EGF-R was immunoprecipitated from mock and GnT-III transfectants of WiDr cells. Tyrosine phosphorylation levels of EGF-R were analyzed by Western blotting using PY20 (upper panel). The membrane was reprobed with a specific antibody to EGF-R to verify that equal amounts of immunoprecipitated proteins were obtained (lower panel). Arrows indicate the proteins of interest. IP indicates immunoprecipitation. Ab, antibody.

Tyrosine Phosphorylation of EGF-R—To verify whether or not GnT-III transfection led to an alteration in EGF-R function, tyrosine phosphorylation levels of EGF-R after EGF treatment were investigated. As shown in Fig. 6, the levels of EGF-R phosphorylation were dramatically enhanced at 5 min after EGF treatment in both mock and GnT-III transfectants of WiDr cells. The time course pattern of EGF-R phosphorylation in GnT-III transfectants was quite similar to that of mock transfectants, suggesting that a similar signaling via EGF-R was occurring (Fig. 8, A and B).

Tyrosine phosphorylation pattern of α-Catenin after EGF stimulation. As shown in Fig. 5, the pattern of tyrosine phosphorylation of α-Catenin after EGF stimulation was investigated as described in Fig. 5. Lane 1 indicates WiDr-M1, lane 2 indicates WiDr-GT1, lane 3 indicates c-srcY527F transfected WiDr-M1, lane 4 indicates c-srcY527F transfectected WiDr-GT1, and lane 5 indicates c-srcY527F-transfectected WiDr-GT2. B, after 48 h of the transfaction, cells were treated with tunicamycin for 3 h and collected. Tyrosine phosphorylation of α-Catenin was investigated as described in Fig. 5. Lane 1 indicates c-srcY527F-transfectected WiDr-M1, lane 2 indicates WiDr-M1, and lane 3 indicates c-srcY527F-transfectected WiDr-M1 with tunicamycin treatment.

DISCUSSION

In many glycoproteins, particularly adhesion molecules and growth factor receptors on cell surfaces, N-linked oligosaccharides contribute to the folding, stability, and biological functions of the molecules (31–33). Our results indicate that the overexpression of GnT-III could lead to the modification of the tyrosine phosphorylation pattern of β-catenin after EGF stimulation. Previous studies from our laboratory have demonstrated that the amount of E-cadherin expression was increased in GnT-III-transfected mouse melanoma B16-hm cells, due to delayed turn-over, compared with mock transfectants (3). Increases in E-cadherin levels on the cell surface enhanced cell-cell contacts and aggregations.

However, in human cancer cells such as WiDr and Huh7, the expression of E-cadherin in GnT-III-transfectants was equivalent to that of mock transfectants, although the oligosaccharide structures of E-cadherin were altered. Although morphological alteration such as compact organization was observed in GnT-III-transfected B16-hm cells, it was not observed in the cases of WiDr and Huh7 cells. These results suggest that proteases,
which contribute to the cleavage of E-cadherin, are different between humans and mice. In contrast, both E4-PHA binding and the molecular size of EGF-R in these transfectants were changed, but the tyrosine phosphorylation of EGF-R on GnT-III transfectants was observed to occur normally both in WiDr and Huh7 cells. Therefore, changes in the tyrosine phosphorylation of β-catenin are not due to EGF-R dysfunction but, rather, its induction by other factors such as downstream signals of EGF-R.

We next examined changes in the integrity of cell-cell contacts between mock and GnT-III-transfected WiDr cells that had been treated with EGF. The GnT-III transfectants of WiDr cells rarely changed in terms of cell morphology and cell-cell contact after EGF stimulation. Interestingly, changes in the cell-cell contacts of mock cells were consistent with the tyrosine phosphorylation pattern of β-catenin. To further explore the relationship between cell-cell adhesion and tyrosine phosphorylation of β-catenin, the localization of β-catenin in mock and GnT-III transfectants of WiDr cells was investigated via an immunofluorescence method. The localization of β-catenin in mock transfectants was redistributed from the lineage of cell membrane to cytoplasm after EGF treatment. In contrast, the localization of β-catenin in GnT-III transfectants remained along the cell-cell contact region of adjacent-adhering cells after EGF treatment. Thus, the nature of cell-cell contacts for the GnT-III-transfected WiDr cells remained unchanged after EGF stimulation.

An alteration in cadherin-mediated cell-cell adhesion is frequently associated with the tyrosine phosphorylation of β-catenin. This phosphorylation is thought to be caused by the Src family of tyrosine kinases and receptors with tyrosine kinase activity such as growth factors like EGF, hepatocyte growth factor, platelet-derived growth factor, and colony stimulating factor-1. In this study, EGF stimulation and src transfection were used to phosphorylate β-catenin. The tyrosine phosphorylation of β-catenin after EGF treatment was suppressed in GnT-III transfectants of three types of cancer cells. Changes in the phosphorylation pattern were a little different in B16-hm cells but remained unchanged in GnT-III transfectants of WiDr and Huh7 cells. Nevertheless, the tyrosine phosphorylation of β-catenin was suppressed in all GnT-III transfectants, suggesting that aberrant glycosylated E-cadherin is not involved in cell-cell contacts. In fact, it has been reported that E-cadherin oligosaccharides are not important for their function as an adhesion molecule (34). However, the present study indicates that bisecting GlcNAc residues on E-cadherin affect intracellular signaling, such as β-catenin phosphorylation, which might be due to an alteration in the E-cadherin-catenin complex. The up-regulation of src or EGF signaling occurs during the progression of cancer (35). If the action of src or EGF to phosphorylate β-catenin is inhibited by GnT-III in vivo, GnT-III contributes to the progression of cancer in multi-steps. In summary, the addition of bisecting GlcNAc residues of E-cadherin-β-catenin by GnT-III down-regulates the tyrosine phosphorylation of β-catenin, which might lead to the suppression of tumor progression such as invasion or metastasis.

REFERENCES

1. Taniguchi, N., Yoshimura, M., Miyoshi, E., Ibara, Y., Nishikawa, A., and Fujii, S. (1996) Glycoconjugate Res. 6, 691–694
2. Dwek, R. A., Christopher, J. E., David, J. H., and Mark, R. W. (1993) Annu. Rev. Biochem. 62, 65–100
3. Yoshimura, M., Ibara, Y., Matsuhashi, M., and Taniguchi, N. (1996) J. Biol. Chem. 271, 13811–13815
4. Tsuda, T., Ikeda, Y., and Taniguchi, N. (1994) (2000) J. Biol. Chem. 278, 21988–21982
5. Takeichi, M. (1991) Science 251, 1451–1455
6. Vlieghe, K., Vakal, J. A., and Fiers, W., and Van Roy, F. (1991) Cell 66, 105–117
7. Frixen, U. H., Behrens, J., Sachs, M., Eberle, G., Voss, B., Warden, A., Kochner, D., and Birchmeier, W. (1991) J. Cell Biol. 113, 173–185
8. Umbas, R., Schalken, J. A., Aalders, T., Carter, B. S., Karhaus, H. F. M., Schafaima, H., Debruyne, F. M. J., and Jaques, W. B. (1992) Cancer Res. 52, 5104–5109
9. Mayer, G., Johnson, J. P., Leitl, F., Jauch, K. W., Heins, M. M., Schildberg, F. W., Birchsmeier, W., and Funk, I. (1993) Cancer Res. 53, 1690–1695
10. Dorudi, S., Sheffield, J. P., Poulson, R., Northover, J. M. A., and Hart, I. B. (1993) Am. J. Pathol. 142, 981–986
11. Narasimham, S. (1982) J. Biol. Chem. 257, 10235–10242
12. Yoshimura, M., Nishikawa, A., Ibara, Y., Taniguchi, S., and Taniguchi, N. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8754–8758
13. Nagafuchi, A., and Takeichi, M. (1988) EMBO J. 7, 3679–3684
14. Ozawa, M., Barabashult, H., and Kemler, R. (1989) EMBO J. 8, 1711–1717
15. Oya, M., Kanai, Y., Ochiai, A., Akimoto, S., Oda, T., Yanagihara, K., Nagafuchi, A., Tsukita, S., Shihabato, S., Ito, F., Takeichi, M., Matsuda, H., and Hirohashi, S. (1994) Cancer Res. 54, 6282–6287
16. Kawanishi, J., Kato, J., Sasaki, K., Fujii, S., Watanabe, N., and Niitsu, Y. (1995) Mol. Cell. Biol. 15, 1175–1181
17. Behrens, J., Vakat, L. F., Fris, R., Winterhager, E., Roy, F. V., Maree, M. M., and Birchmeier, W. (1995) J. Cell Biol. 120, 775–784
18. Sommers, C. L., Geinman, E. P., Kemler, R., Cowin, P., and Byers, S. W. (1994) Cancer Res. 54, 3544–3552
19. Muller, T., Chondas, A., Reichmann, E., and Ullrich, A. (1999) J. Biol. Chem. 274, 10173–10183
20. Takahashi, K., Suzuki, K., and Tsukutani, Y. (1997) Oncogene 15, 71–78
21. Hamaguchi, M., Matsuhashi, N., Ohnishi, Y., Gotoh, B., Takeichi, M., and Nagai, Y. (1993) EMBO J. 12, 307–314
22. Hoschuetzky, H., Acher, H., and Kemler, R. (1994) J. Cell Biol. 127, 1375–1380
23. Hazan, R. B., and Norton, L. (1998) J. Biol. Chem. 273, 9078–9084
24. Ibara, Y., Nishikawa, A., Okuda, T., and Nishikawa, M., Nishikawa, A., and Taniguchi, N. (1993) J. Biochem. (Tokyo) 113, 692–698
25. Niwa, H., Yamamura, K., and Miyazaki, J. (1991) Gene 106, 193–200
26. Taniguchi, N., Nishikawa, A., Fujii, S., and Gu, J. (1989) Methods Enzymol. 179, 397–408
27. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
28. Miyoshi, E., Nishikawa, A., Ibara, Y., Gu, J., Sugiyama, T., Hayashi, N., Fusamoto, H., Kamada, T., and Taniguchi, N. (1995) Cancer Res. 53, 3899–3902
29. Yamashita, K., Hirot, A., and Kobata, A. (1983) J. Biol. Chem. 258, 14753–14755
30. Nada, S., Okuda, M., MacAuley, A., Cooper, J. A., and Nakagawa, H. (1991) Nature 351, 69–72
31. Hakomori, S-I. (1989) Adv. Cancer Res. 52, 257–331
32. Dwek, R. A. (1995) Science 269, 1224–1225
33. Wynn, D., F., Choi, J. S., Li, J., Knoppers, M. H., Willis, K. J., Arulananandam, A. R. N., Smolyar, A., Reinherz, E. L., and Wagner, G. (1995) Science 269, 1273–1278
34. Shirahashi, Y., Nose, A., Iwasaki, K., and Takeichi, M. (1986) Cell Struct. Funct. 11, 245–252
35. Biscardi, J. S., Tice, D. A., and Parsons, S. J. (1999) Adv. Cancer Res. 76, 61–119