Unusually High Expression of \(N\)-Acetylglucosaminyltransferase-IVa in Human Choriocarcinoma Cell Lines: A Possible Enzymatic Basis of the Formation of Abnormal Biantenary Sugar Chain

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**ABSTRACT**

Structural analysis of the sugar chains of human chorionic gonadotropin (hCG) has revealed that abnormal biantenary structure appears specifically on hCG in the urine of chorion carcinoma patients. However, the enzymatic and molecular mechanisms of the biosynthesis of abnormal biantenary sugar chains have not yet been elucidated. In this report, the enzyme activities and the expression levels of mRNAs of \(N\)-acetylgucosaminyltransferases (GnT-I) to -V, \(\beta\)-1,4-galactosyltransferase, and \(\alpha\)-mannosidase II in normal human placentae and three human chorion carcinoma cell lines were investigated. Gnt-IV activities in chorion carcinoma cell lines were increased from 16- to 66-fold and Gnt-III activity was increased from 15- to 25-fold as compared with those in human placentae, whereas other enzyme activities were not increased significantly. The mRNA expression levels generally correlated with their enzyme activities. Among the two Gnt-IV genes found in human tissues only Gnt-IVa gene was strongly expressed in the cancer cells: from three to seven times as much as in the normal tissue, whereas that of Gnt-IVb remained constant. On the basis of these results, we proposed that ectopic expression of Gnt-IVa gene should occur along with the malignancy of trophoblastic tissues, and that the increased Gnt-IV activity should be the main cause of the formation of abnormal biantenary sugar chains in chorion carcinoma. A possible enzymatic basis of the biosynthesis of abnormal biantenary sugar chains is discussed.

**INTRODUCTION**

hCG is a glycoprotein hormone produced by normal trophoblast cells of the placenta during pregnancy. It is also produced by the cells of various trophoblastic diseases such as hydatidiform mole, invasive mole, and chorion carcinoma (1–5). This hormone is essential for the maintenance of the fetus during the first trimester of pregnancy and also stimulates steroidogenesis and cyclic AMP production in rat testicular tissues (6, 7). hCG is a heterodimer composed of \(\alpha\)- and \(\beta\)-subunits, similar to the three mammalian pituitary glycoproteins: luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone (8). Each subunit of hCG contains two asparaginylmannoside linked sugar chains, and the \(\beta\)-subunit also contains four serine-linked sugar chains. These sugar chains play important roles in expressing the biological activity of hCG (9, 10).

Comparative studies of the oligosaccharides that are released by hydrazinolysis from hCG samples purified from the urine of pregnant women and patients with trophoblastic diseases revealed that extensive structural alteration exists in the asparagine-linked sugar chains of tumor hCGs (11). hCGs that are obtained from pregnant women and patients with hydatidiform mole contain the sialylated forms of oligosaccharides A (without the Fuc \(\alpha\)-1–6 residue) and B in Fig. 1. The hCGs from patients with invasive mole contain the sialylated forms of oligosaccharides A, B, and D. The hCGs from patients with chorion carcinoma contain either sialylated or nonsialylated forms of all of the eight oligosaccharides shown in Fig. 1. These results indicated that an abnormal expression of Gnt-IV is the key to alter the glycosylation of hCG in the malignant trophoblastic diseases. Because oligosaccharides C and D were not detected in the hCGs from pregnant women and patients with hydatidiform mole, Gnt-IV, which catalyzes the formation of the GlcNAc\(\beta\)-1–4 Man\(\alpha\)-1–3 group, should not be expressed in their hCG producing cells. Presence of oligosaccharides D in the hCGs from invasive mole patients indicated that the enzyme is abnormally expressed in this disease. Expression of oligosaccharides C together with oligosaccharides D in the hCGs from chorion carcinoma indicated that the abnormally expressed Gnt-IV in chorion carcinoma can act on monoantennary sugar chains as well as on biantenary sugar chains. It was reported by Gleeson and Schachter (12) that Gnt-IV solubilized from the Golgi membrane can use monosialylated sugar chains as acceptors. However, oligosaccharide C has not been detected in the glycoproteins produced by various normal cells. Hence, we called them “abnormal biantenary sugar chains,” expecting them to become important tumor markers in the future. Actually, the abnormal biantenary sugar chains were later found in the \(\gamma\)-glutamyltransferase purified from human hepatoma (13) and in the carcinoembryonic antigen obtained from colon cancer (14). Therefore, it is important to investigate the control mechanism to prevent formation of the abnormal biantenary sugar chains in normal cells.

We have previously suggested (15) that the following two steps will induce the altered glycosylation of hCG in tumor cells: (a) ectopic expression of the regular Gnt-IV; and (b) modification of the substrate specificity of the enzyme. However, the detailed biosynthetic mechanism to form the abnormal biantenary sugar chains remained to be elucidated.

Recently, we have established a sensitive assay method for Gnt-IV (16). By using this method, we successfully purified Gnt-IVa from bovine small intestine (17), and cloned its cDNA (18). We also succeeded in cloning the cDNAs of human Gnt-IV (19, 20). Unlike other GnTs, mammalian Gnt-IV gene constructs an active gene family consisting of Gnt-IVa and Gnt-IVb genes (19, 20). Although precise enzymatic differences between the two gene products are under investigation, it is quite interesting to know which gene product should...
be the cause to form the abnormal biantennary sugar chains. In this study, we tried to ascertain the enzymatic and genomic background of the formation of abnormal biantennary sugar chains by analyzing and comparing related enzyme activities and their mRNA expression levels between choriocarcinoma cell lines and a normal placenta.

MATERIALS AND METHODS

Cell Lines and Tissue. The human choriocarcinoma cell line BeWo was obtained from Health Science Research Resources Bank (Osaka, Japan). Human choriocarcinoma cell lines JAR and JEG-3 were purchased from American Type Culture Collection (Rockville, MD). Human normal placenta were kindly donated by Dr. S. Tagami (Hokkaido University, Sapporo, Japan). BeWo cells were maintained in Ham’s F-12 Kaighn’s modification medium (Life Technologies, Inc.) supplemented with 15% FBS (HyClone). JAR cells were grown in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% FBS, 10 mM HEPES, 1 mM sodium pyruvate, and 2.5 mg/ml glucose. JEG-3 cells were cultivated in MEM (Life Technologies, Inc.) supplemented with 10% FBS. All of the media mentioned above were supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin, and all of the cell lines were incubated at 5% CO₂ humidified atmosphere at 37°C. Seventeen cell lines used to examine the averages of enzyme activities were: (a) Bowes (malignant melanoma); (b) A-549 (lung carcinoma); (c) YMB-1 (breast cancer); (d) HepG2 (hepatocellular carcinoma); (e) HuO-3N1 (osteosarcoma); (f) CACO-2 (adenocarcinoma, colon); (g) HeLaS3 (cervical carcinoma); (h) MOLT-4 (T-cell leukemia); (i) JAR cells (placenta choriocarcinoma); (j) HuO-3N1 (osteosarcoma); (k) JEG-3 cells (human chorionic carcinoma); (l) HeLaS3 (cervical carcinoma); (m) MOLT-4 (T-cell leukemia); (n) EoL-1 (lymphoblastoid leukemia); (o) MOL-M14 (T-cell leukemia); (p) EoL-1 (lymphoblastoid leukemia); (q) HuO-3N1 (osteosarcoma); (r) HeLaS3 (cervical carcinoma); (s) JEG-3 cells (human chorionic carcinoma); (t) MOLT-4 (T-cell leukemia); (u) EoL-1 (lymphoblastoid leukemia). The human choriocarcinoma cell line BeWo was used as a model of choriocarcinoma tissue. Compared with the values of GnT-I and -II activities in seventeen human cell lines [3,130–33,720 (mean, 13,260) pmol/h/mg protein and 3,230–15,820 (mean, 9,510) pmol/h/mg protein, respectively], normal placenta have high GnT-I activity (25,340 pmol/h/mg protein) but contain low GnT-II activity (1,670 pmol/h/mg protein). Possibly, the low GnT-II activity is the enzymatic basis of the formation of monoantennary sugar chain found in the hCG produced by a normal placenta. GnT-IV activities in choriocarcinoma cells were increased from 16- to 66-fold over those in normal placentae. The GnT-IV activities of these cancer...
cells (1,920 to 7,930 pmol/h/mg) are the highest among the various human cancer cell lines investigated in our laboratory (the values of the 17 human cell lines were 80–5,210 pmol/h/mg protein, mean = 750 pmol/h/mg protein). Such a strong activity of GnT-IV should affect the branch formation of sugar chains in these cancer cells.

GnT-III activities in the choriocarcinoma cell lines were also drastically increased as compared with those of normal placenta. hCG obtained from culture medium of BeWo cells was reported to have bisected complex-type sugar chains, which are the products of GnT-III (24). However, such structures were not detected at all in the hCGs obtained from the urine of choriocarcinoma patients nor of healthy individuals. Therefore, the observed augmentation of GnT-III activity may be an independent phenomenon from carcinogenesis in trophoblastic tissues.

Activities of the other enzymes were not increased significantly in the choriocarcinoma cells when compared with normal placentae. Therefore, the major difference in the enzymes involved in the formation of abnormal biantennary sugar chains between normal placentae and the choriocarcinoma cells is the extraordinarily increased GnT-IV activity.

Northern Blot Analysis of mRNAs for the Enzymes Related to the Branch Formation of Asparagine-linked Sugar Chains in a Normal Placenta and in Choriocarcinoma Cell Lines. Compared with the rather similar patterns of the enzyme activities among the three choriocarcinoma cells, the pattern of mRNA expression levels of the enzymes in each cell line is quite different (Fig. 3). In JAR cells, only GnT-IVa mRNA was present in an increased concentration. In BeWo cells, the mRNAs of GnT-III and GnT-IVa were extremely enhanced. In any case, the mRNA of GnT-IVa, but not of GnT-IVb was strongly expressed in all of the three choriocarcinoma cell lines.

The mRNA level of each enzyme shown in Fig. 3 was generally correlated to the actual enzyme activity (Fig. 2), although there were slight discrepancies in the case of α-Man′ase II in JAR cells and GnT-I in the three cell lines for unknown reasons. Although G3PDH is generally used as a control for measuring mRNA expression level, it could be overexpressed in established cell lines. Therefore, we used the β-actin mRNA to normalize the expression levels of glycosyltransferases and glycosidase mRNAs.

DISCUSSION

The data reported in this study indicated that the enzymatic basis of the altered glycosylation found in tumor hCG is not simple, as we discussed previously (15).

Current knowledge of the biosynthesis of the complex type sugar chains is as follows. The glucosylated high mannose type sugar chains, added cotranslationally to a nascent polypeptide chain, are processed to Man₆GlcNAc₂ when the glucosylated polypeptide is transported to the Golgi apparatus. When the glycopeptide reaches the medial Golgi, GlcNAcβ₁–2 is added to the Man₆–1 arm of this heptasaccharide by the catalytic action of GnT-I. Then the two α-mannosyl residues are removed from the Man₆–1–6 arm by the action of α-Man′ase II to form the agalacto-monoantennary sugar chain (pathway I in Fig. 4). More highly branched complex-type sugar chains are then formed from this monoantennary sugar chain as follows. Agalacto-biantennary sugar chain will be formed by the action of GnT-II (pathway II). Then the 2,4-branched triantennary sugar chain will be formed by the catalytic action of GnT-IV (pathway V).

As described in “Results,” normal placenta and choriocarcinoma cell lines contain lower level of GnT-II activity as compared with other human cell lines. Such a low GnT-II activity could be the basis of the formation of monosaccharide oligosaccharide (oligosaccharide A without the Fuc₁–6 residue in Fig. 1), which occupies approximately one-fourth of all of the sugar chains of the hCGs from pregnant women (25). It was confirmed that a small amount of GnT-IV activity is expressed in placenta. However, the sugar patterns of hCGs from pregnant women indicated that the activity of GnT-IV is not strong enough to produce triantennary sugar chains.

In choriocarcinoma cells, in which GnT-IV activity is remarkably increased, an additional pathway may work as shown in Fig. 4. The enzyme level is strong enough to form 2,4-branched triantennary sugar chains (pathway V). On the basis of the report of Gleeson and Schachter (12) and ourselves (17), the highly expressed GnT-IV may also work on the agalacto-monoantennary sugar chain to form an abnormal biantennary sugar chain (pathway IV). By using a hen...
UP-REGULATION OF GnT-IVa IN CHORIOCARCINOMA CELLS

Fig. 4. Possible mechanism of the formation of various sugar chain structures in choriocarcinoma hCG. M, Man, GN, GlcNAc; R, GlcNAcβ1–4GlcNAc-Asn.

oiduct membrane preparation as an enzyme source, Allen et al. (26) found that the pathways III and VI in Fig. 4 do occur to form the agalacto abnormal biantennary sugar chains. However, these pathways seem to unlikely to work in choriocarcinoma inasmuch as no intermediate hybrid-type oligosaccharide was detected in the urinary hCGs. Therefore, we concluded that pathway IV is the most likely one to form the abnormal biantennary sugar chains in choriocarcinoma cells. By in vitro experiments, we found that GnT-II can act on the agalacto abnormal biantennary sugar chains to form triantennary sugar chains (pathway VII). If pathway VII actually works in vivo, certain amounts of the triantennary sugar chains will be produced from the accumulated abnormal biantennary sugar chains, even if the normal pathway (pathways II and V) becomes restricted. Actually, from two to four times more triantennary sugar chains (structures D in Fig. 1) than biantennary ones (structures B) were detected in the hCGs from choriocarcinoma patients (25), which is hard to explain without the contribution of the pathway VII.

The evidence that no abnormal biantennary sugar chain was detected in the hCGs from patients with invasive mole, despite having triantennary sugar chains, may well be explained by competition between GnT-II and GnT-IV for the common substrate, monoaentary structure. Although the weak GnT-II activity in normal placenta seems dominitive against weaker GnT-IV activity in the urine of a choriocarcinoma patient. It was later explained, however, that the decrease of the hormonal activity was caused by the loss of sialic acid residues (27). No precise investigation has been performed thus far for the change in in vitro activity of hCG with sugar chains of abnormal branching structures. We would like to examine the relation of in vitro activity and branch structures of hCG in the future.

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