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Biochimica et Biophysica Acta 1455 (1999) 315–326

BIOCHIMICA ET BIOPHYSICA ACTA

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Review

Structure, pathology and function of the N-linked sugar chains of human chorionic gonadotropin

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Received 13 October 1998; received in revised form 1 April 1999; accepted 1 April 1999

Abstract

Human chorionic gonadotropin (hCG) contains five acidic N-linked sugar chains, which are derived from three neutral oligosaccharides by sialylation. Each of the two subunits (hCGα and hCGβ) of hCG contain two glycosylated Asn residues. Glycopeptides, each containing a single glycosylated Asn, were obtained by digestion of hCGα with trypsin, and of hCGβ with chymotrypsin and lysyl endopeptidase. Comparative study of the sugar chains of the four glycopeptides revealed the occurrence of site-directed glycosylation. Studies of the sugar chains of hCGs, purified from urine of patients with various trophoblastic diseases, revealed that choriocarcinoma hCGs contain sialylated or non-sialylated forms of eight neutral oligosaccharides. In contrast, hCGs from invasive mole patients contain sialyl derivatives of five neutral oligosaccharides. The structural characteristics of the five neutral oligosaccharides, detected in choriocarcinoma hCGs but not in normal placental hCGs, indicate that *N*-acetylglucosaminyltransferase IV (GnT-IV) is abnormally expressed in the malignant cells. This supposition was confirmed by molecular biological study of GnT-IV in placenta and choriocarcinoma cell lines. The appearance of tumor-specific sugar chains in hCG has been used to develop a diagnostic method of searching for malignant trophoblastic diseases. In addition, a summary of the current knowledge concerning the functional role of N-linked sugar chains in the expression of the hormonal activity of hCG has been presented.

Keywords: Human chorionic gonadotropin; N-Linked sugar chains; *N*-Acetylglucosaminyltransferase IV; Choriocarcinoma; Hydatidiform mole; Invasive mole

Contents

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0925-4439/99/$ – see front matter
PII: S0925-4439(99)00060-5

1. Introduction

Human chorionic gonadotropin (hCG) is a glycoprotein hormone, which is produced by the syncytiotrophoblast cells of human placenta, and is involved in the maintenance of the corpus luteum in early pregnancy. It is a heterodimer composed of subunits designated hCGα and hCGβ. HCGα is *N*-glycosylated at Asn-52 and Asn-78 [1]. HCGβ also contains two N-linked sugar chains at Asn-13 and Asn-30, and four O-linked sugar chains at Ser-121, Ser-127, Ser-132 and Ser-138 [2].

2. N-Linked oligosaccharides of hCG

The structures of the N-linked sugar chains of hCG were first reported in 1979 [3,4]. The establishment of hydrazinolysis [5], a chemical means of releasing the N-linked sugar chains from glycoproteins as oligosaccharides, was of key importance in enabling us to obtain reliable quantitative information as to the glycosylation patterns of various glycoproteins. As shown in Fig. 1A, fractionation of the desialylated oligosaccharide fraction obtained from the hydrazinolysis of hCG produced three major peaks. Structural study of the oligosaccharides in peaks I, II and III revealed that they are N5, N6 and N8 shown in Fig. 2, respectively [3]. At first, the data were considered to reflect the microheterogeneity of sugar chains, which had been widely found in the sugar chains of various glycoproteins. However, comparative study of the sugar patterns of hCGα and hCGβ revealed that site-specific glycosylation occurs in the N-linked sugar chains of hCG. As shown in Fig. 1B,C, only N6 and N8 were detected from the hCGα sample, while N5 and N6 were detected from the hCGβ sample, in approximately equal amounts [6]. Using one- and two-dimensional ^{1}H-NMR spectroscopy, Weisshaar et al. [7] investigated the site-specific *N*-glycosylation of hCG. They found that the main oligosaccharide attached to Asn-52 of hCGα was sialylated N8. Approx. 60% of the oligosaccharides attached to Asn-78 of hCGα were sialylated N6 and the remainder was sialylated N8. N5 and N6, in the molar ratio of 1:3, were found to be linked to the Asn-13 of hCGβ, while mostly sialylated N5 was found to be attached to the Asn-30 of hCGβ. Amano and Kobata [8] confirmed their finding by investigating the behaviors of four glycopeptides obtained from hCGα and hCGβ on immobilized *Aleuria aurantia* lectin and Concanavalin A columns.

These data indicate that even the N8 of hCG should not be considered an incomplete biosynthetic product, but a final product at a particular site of the hCG molecule.

The specific distribution of different sugar chains in the two subunits of hCG suggests that the three-dimensional structure of the polypeptide portion of hCG controls the maturation of the sugar moieties linked to it. Study of the N-linked sugar chains of the free hCGα supports this assumption. Together with dimeric hCG, a very small amount of free hCGα can be detected in the urine of pregnant women. In spite of having the same amino acid sequence as the hCGα isolated from heterodimeric hCG, this free hCGα cannot bind to hCGβ [9]. Parsons and Pierce [10] and Cole et al. [11] suggested that unusual *O*-glycosylation of the free hCGα may prevent its association with hCGβ. However, Kawano et al. [12] reported that no O-linked sugar chain was detected in their free hCGα preparation. Instead, they found that the subunit contains only one glycosylated Asn, and the structures of the sugar chains are a mixture of sialylated N5 and N6. This result indicates that sialylated N5 on the free hCGα may sterically inhibit association with hCGβ. In due con-

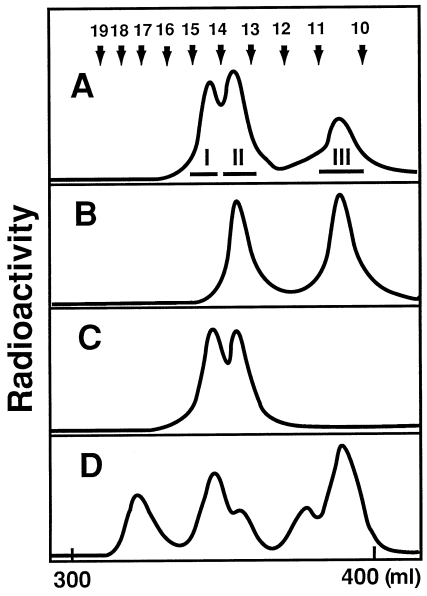

Fig. 1. Fractionation of desialylated radioactive oligosaccharide fractions by Bio-Gel P-4 (under 400 mesh) column. Arrows indicate the elution positions of glucose oligomers (numbers indicate the glucose units). (A) Oligosaccharide fraction from urinary hCG obtained from a pregnant woman; (B) fraction from hCGα of placental hCG; (C) fraction from hCGβ of placental hCG; (D) fraction from choriocarcinoma hCG.

sideration of the biosynthetic mechanism of N-linked sugar chains, these data can be interpreted as follows.

Two tetradecasaccharides (Glc_3·Man_9·$GlcNAc_2$) are added co-translationally to both hCGα and hCGβ while they are being translated in the rough endoplasmic reticulum of syncytiotrophoblast. It was found that the two subunits are associated before they are transferred to the Golgi apparatus [13]. Therefore, the four N-linked sugar chains present in the early stages of the heterodimer production should be a series of high mannose-type sugar chains, which will be processed to Man_5·$GlcNAc_2$ during the heterodimer's transport to the medial Golgi. After reaching the medial Golgi, the heterodimer's N-linked sugar chains will be processed by the concerted action of β-*N*-acetylglucosaminyltransferases I and II, α-mannosidase II, β-galactosyltransferase, and sialyltransferase [14]. However, the maturation of the four N-linked sugar chains is controlled by the steric interactions of the two subunits, producing the site-specific complex-type sugar chains at the four *N*-glycosylation sites. Possibly, the small amount of free hCGα produced is a result of the failure of the peptide to accept one tetradecasaccharide in the rough endoplasmic reticulum of the syncytiotrophoblast, probably because folding of the polypeptide moiety hides one of the two potential *N*-glycosylation sites of the hCGα molecule, as found in the case of ovalbumin [15]. This hCGα with only one N-linked sugar chain may not be able to combine with hCGβ, and is transferred to Golgi as free hCGα. Since the maturation of its N-linked sugar chain is not controlled by the steric effect of hCGβ, it will acquire the largest complex-type sugar chain that can be formed by the glycosylation machinery of syncytiotrophoblast.

The theory that the larger N-linked sugar chains of free hCGα inhibit its binding with hCGβ was proposed by Blithe [16]. She found that a free hCGα sample, purified from the culture medium of JEG-3 cells, contained larger complex-type sugar chains than that dissociated from intact hCG molecules purified from the same medium. This free hCGα also cannot combine with hCGβ to form the dimeric hormone. However, the free hCGα, purified from the culture medium of JEG-3 cells grown in the presence of swainsonine, could combine with hCGβ. Virtually all N-linked sugar chains of the free hCGα from swainsonine-treated cells were released by endo-β-*N*-acetylglucosaminidase H digestion, indicating that they are either high mannose-type or hybrid-type sugar chains. Therefore, it was estimated that the complex-type sugar chains of the free hCGα sterically inhibit its binding to hCGβ. Peters et al. [17] reported that only 10% of the free hCGα secreted by explants of 10 week old placenta are *O*-glycosylated, and also indicated that *O*-glycosylation of hCGα is a late event in the secretory pathway of syncytiotrophoblast cells, as compared to the rapid combination of hCG subunit precursors to form an αβ dimer in the rough endoplasmic reticulum. Based on these data, they concluded that *O*-glycosylation of the hCGα could not be the cause of production of free hCGα.

3. HCGs in trophoblastic diseases

A large amount of hCG is detected in the sera and the urine of patients with various trophoblastic diseases [18]. The major trophoblastic diseases are a

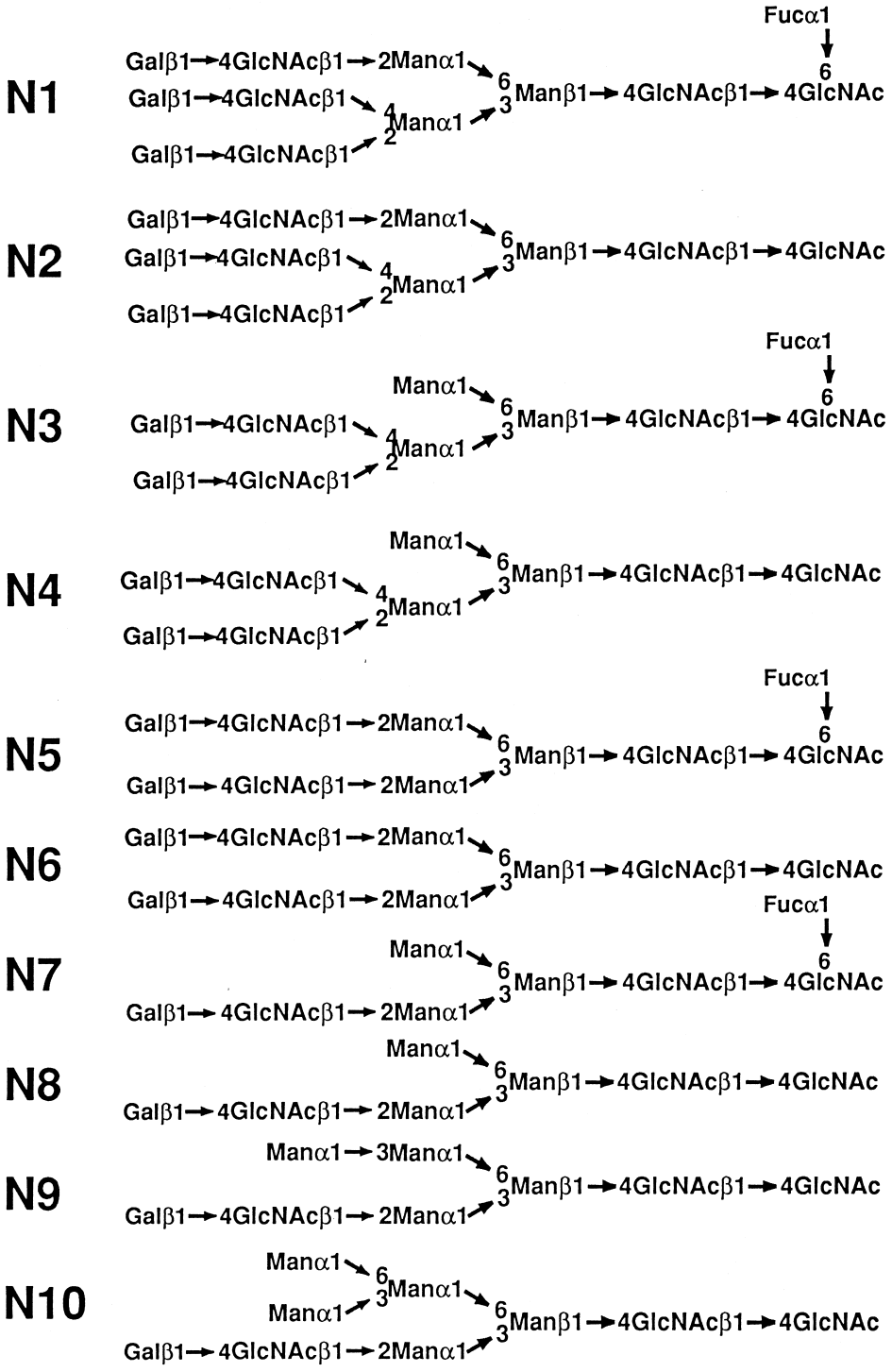

Fig. 2. Structures of desialylated N-linked sugar chains isolated from various hCG preparations.

hydatidiform mole, an invasive mole, and choriocarcinoma. Hydatidiform moles are considered a benign lesion. However, some of the hydatidiform moles apparently show more malignant characteristics, such as invasion into the surrounding tissues and metastasis, and are discriminated as an invasive mole from most typical moles. On the other hand, patients with choriocarcinoma show definitive fea-

tures of a malignant tumor. Although prophylactic chemotherapy is effective in retarding the development of persistent gestational trophoblastic diseases such as invasive mole and choriocarcinoma, use of chemotherapy at the time of molar evacuation is controversial because of the drug toxicity. Accordingly, precise diagnosis of the patients with these three diseases is clinically important for the treatment of patients with trophoblastic diseases.

It has been known for the past three decades that altered glycosylations occur in the glycoproteins produced by various tumor cells [19]. Because constant N-linked sugar chain patterns were obtained from the urinary hCGs of healthy pregnant women, it was considered that any structural change, induced in the sugar chains of hCG produced by choriocarcinoma, may be useful for developing a diagnostic method for malignant trophoblastic diseases. An hCG sample, purified from the urine of a patient with choriocarcinoma, was shown to have quite a different sugar pattern from those of normal pregnant women [20]. First of all, the oligosaccharide fraction obtained by hydrazinolysis from the choriocarcinoma hCG was almost free from acidic oligosaccharides. When subjected to Bio-Gel P-4 column chromatography, the oligosaccharide fraction gave the elution pattern as shown in Fig. 1D. Structural study of the oligosaccharides included in the five peaks revealed that eight neutral oligosaccharides, N1–N8 as shown in Fig. 2, occur in the choriocarcinoma hCG. Further study of the sugar chains of hCGs, purified from the urine of three additional patients with choriocarcinoma, indicates that the deletion of sialic acids is not present in all choriocarcinoma hCG [21]. However, the eight oligosaccharides, N1–N8, were detected in all three choriocarcinoma samples after removal of the sialic acid from the sugar chains. The structural change detected in the sugar chains of choriocarcinoma hCG is apparently quite complicated, but it can be reasonably explained by changes in two glycosyltransferases in choriocarcinoma. An increase in the molar ratio of total fucosylated oligosaccharides (N1, N3, N5 and N7 in Fig. 2) indicated that the fucosyltransferase responsible for formation of the Fucα1-6GlcNAc group is highly expressed in choriocarcinoma cells. Appearance of oligosaccharide N7 may indicate that the fucosyltransferase has wider specificity than that in normal syncytiotrophoblasts. Structurally, oligosaccharides N1, N2, N3 and N4 can be formed by addition of the Galβ1-4GlcNAcβ1-4 outer chain to N5, N6, N7 and N8, respectively. Therefore, *N*-acetylglucosaminyltransferase IV (GnT-IV) [14] must be strongly expressed in choriocarcinoma cells.

The N-linked sugar chains of the hCG samples, purified from the urine of three patients with hydatidiform mole, were identified as almost the same oligosaccharide patterns as in the case of non-trophoblastic disease hCG (Fig. 1A). However, examination of the N-linked sugar chains of urinary hCG samples obtained from two patients with invasive mole, provided additional interesting evidence as to the mechanism of altered glycosylation of the tumor hCG [22]. These samples contained the oligosaccharides N1, N2, N5, N6, N7 and N8, but not N3 and N4. Detection of oligosaccharides N1 and N2 in invasive mole indicated that GnT-IV is expressed in these cells. However, absence of oligosaccharides N3 and N4 indicates that the newly expressed GnT-IV can transfer an *N*-acetylglucosamine residue to biantennary complex-type sugar chains, but not to monoantennary sugar chains. Gleeson and Schachter reported that GnT-IV solubilized from Golgi membrane can add a β-*N*-acetylglucosamine residue to monoantennary sugar chains [23]. However, oligosaccharides N3 and N4 have not been detected in the glycoproteins produced by various animal cells. Hence, we called them *abnormal biantennary sugar chains* expecting them to become important tumor markers. A control mechanism to prevent formation of abnormal biantennary sugar chains must exist in the Golgi apparatus of normal cells.

4. Altered expression of GnT-IV in choriocarcinoma cells

GnT-IV is expressed in many animal species [23–27]. However, GnT-IV activities in these cells are generally lower than other β-*N*-acetylglucosaminyltransferases (GnTs) responsible for the formation of complex-type N-linked sugar chains. Oguri et al. [28] recently found that high GnT-IV activity is expressed in bovine small intestine, and successfully purified the enzyme to homogeneity. The substrate specificity of this enzyme was the same as reported

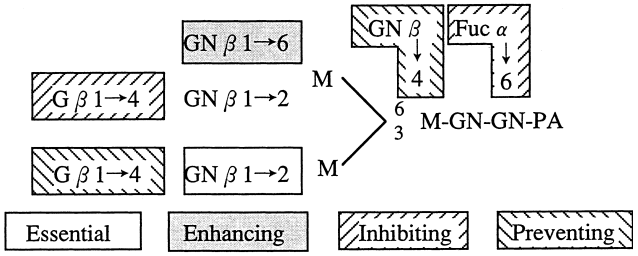

Fig. 3. Substrate specificity of GnT-IV. PA, pyridylamine. The effect of each monosaccharide residue is shown by the pattern of the enclosing box.

by Gleeson and Schachter [23], absolutely requiring the presence of the GlcNAcβ1-2Manα1-3 group in the acceptor oligosaccharide. The presence of the GlcNAcβ1-2Manα1-6 or the GlcNAcβ1-6Manα1-6 groups in the acceptor oligosaccharides is not essential but they are more favorable as substrates (Fig. 3). Cloning of the gene encoding this enzyme revealed that the enzyme has a typical type II membrane-bound protein structure, but has no homology with other cloned GnTs [29].

In order to investigate the enzymatic basis of the altered glycosylation of the hCG produced in choriocarcinoma cells, human cDNA for GnT-IV was obtained from a human liver cDNA library. Surprisingly, two active GnT-IV genes, with 91 and 64% homology to the bovine GnT-IV gene, were obtained [58]. The translation products of these two genes were named GnT-IVa and GnT-IVb, respectively. The cellular expression of the two genes is strikingly different. GnT-IVb gene is expressed in all human organs at almost the same level, while GnT-IVa gene is highly expressed in specific organs such as pancreas, thymus, small intestine, and leukocytes [30].

When activities of the glycosyltransferases related to the formation of the abnormal biantennary sugar chains were compared in normal placenta and several choriocarcinoma cell lines, the GnT-IV activity was strikingly increased in the cancer cells [59]. GnT-III activity was also increased, while GnT-I and -II, β1,4-galactosyltransferase and α-mannosidase II activities were not increased significantly from those of the normal placenta. They also found that GnT-II activity in normal placenta, as well as in choriocarcinoma cells, was lower than in other cells which never produce monoantennary sugar chains.

Northern blot analysis revealed that the GnT-IVa gene was extraordinarily over-expressed in the cancer cells, while the GnT-IVb gene was expressed at the same level as in normal placenta [59]. So far, no difference in the substrate specificities of GnT-IVa and GnT-IVb has been found. Both can produce the abnormal biantennary structures in vitro. The data, so far described, indicate that low GnT-II activity and over-expression of the GnT-IVa gene, and the resulting increase in GnT-IV activity are the enzymatic basis of the formation of the abnormal biantennary sugar chains in choriocarcinoma cells (Fig. 4). However, these conditions do not explain the subtle differences detected in the N-linked sugar chains of choriocarcinoma hCG and invasive mole hCG. Therefore, more detailed studies of the substrate specificity of GnT-IVa, including the kinetic parameters, and investigation of its topology in the Golgi membrane of the cells of the two diseases must be performed.

In view of the findings that abnormal biantennary sugar chains occur in the glycoproteins produced by several other tumor cells [31,32], studies searching for abnormal biantennary sugar chains will produce important information as to the altered *N*-glycosylation in malignant cells.

5. HCG as a diagnostic marker of malignant trophoblastic diseases

As described in the previous section, qualitative alterations are found in the N-linked sugar chains of hCG samples purified from the urine of patients with invasive mole and choriocarcinoma. Because oligosaccharides N1 and N2 in Fig. 2 were detected in invasive mole and choriocarcinoma hCGs but not in normal pregnant hCGs and hydatidiform mole hCGs, any method that can discriminate between the hCG molecules with or without these oligosaccharides could be used for the diagnosis of malignant trophoblastic diseases.

By investigating the behavior of various complex-type oligosaccharides on several immobilized lectin columns, Yamashita et al. [33] found that these oligosaccharides can be separated into three groups by passing through a *Datura stramonium* agglutinin (DSA)-Sepharose column. The oligosaccharides,

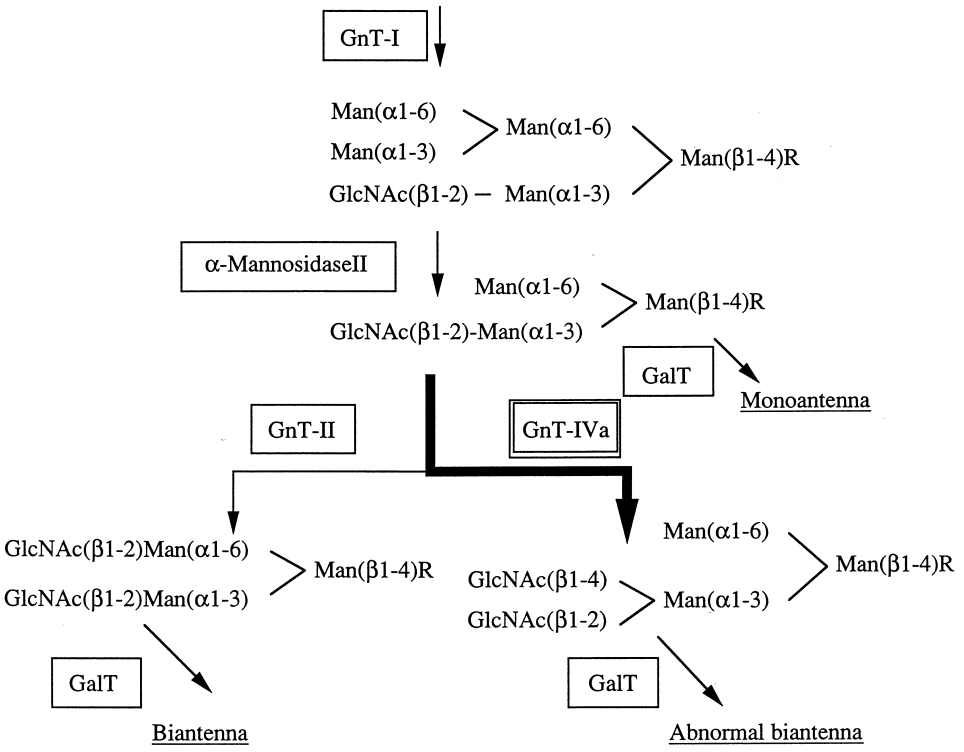

Fig. 4. Deduced mechanism of the abnormal biantennary sugar chain formation in choriocarcinoma cells. In choriocarcinoma cells, the GnT-IVa gene was much more highly expressed than in the normal placenta, which resulted in imbalance between GnT-II and -IV enzyme activities. Bold arrow indicates the increased enzyme activity. R = GlcNAcβ1-4GlcNAc-protein. GalT, β1,4-galactosyltransferase.

which are weakly bound to the column and eluted with buffer, all contain the non-substituted Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Man group. The oligosaccharides, which are strongly bound and then eluted from the column with the elution buffer containing a 1% mixture of β-*N*-acetylglucosamine oligomers, have either the Galβ1-4GlcNAcβ1-6-(Galβ1-4GlcNAcβ1-2)Man group or the Galβ1-4-GlcNAcβ1-3Galβ1-4GlcNAc group as their partial structures. The oligosaccharides having none of these groups pass through the column without any interaction.

Since the binding specificity of the DSA-Sepharose column was expected to be useful for discriminating hCGs with or without oligosaccharides N1 and N2 in Fig. 2, the behavior of urinary hCGs of various trophoblastic diseases on this column was investigated [34]. Almost all hCGs in the urine of a pregnant woman passed through the column without interaction. The elution pattern did not change even after the urine was pretreated by sialidase digestion. In contrast, only a portion of hCG in the urine of a patient with choriocarcinoma passed through the column. The remainder was not eluted even with the elution buffer, but was completely recovered by elution with 0.1 N acetic acid. This unexpectedly strong binding might have occurred because the hCG molecule contains at least two oligosaccharides with the Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)-Man group. The elution step with the buffer containing a 1% mixture of β-*N*-acetylglucosamine oligomers was then omitted, and the amounts of hCG in the two fractions obtained by elution with simple buffer and with 0.1 N acetic acid were measured to determine the percentage of hCG with the Galβ1-4-GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Man group in their sugar chains. As shown in Fig. 5, the values for normal pregnant women and for patients with hydatidiform mole were less than 15%, and did not increase after sialidase digestion. The values for patients with invasive mole were also small, but sialidase treated samples showed much higher values.

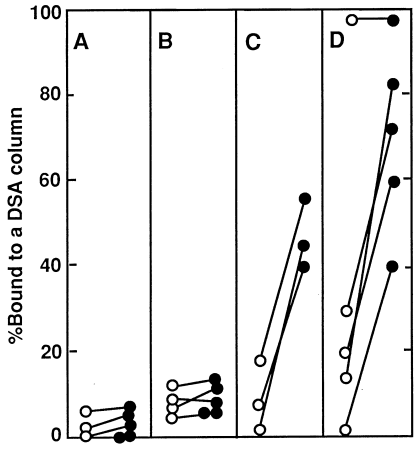

Fig. 5. Percent molar ratio of urinary hCGs bound to a DSA-Sepharose column before (○) and after (●) sialidase digestion. (A) Urine samples from normal pregnant women; (B) those from patients with hydatidiform mole; (C) those from patients with invasive mole; (D) those from patients with choriocarcinoma. Taken from [34].

The results for patients with choriocarcinoma varied more than the others. Some of them behaved very similar to invasive mole hCGs. However, one of the choriocarcinoma hCGs bound completely to the column without sialidase treatment. Therefore, affinity column chromatography with use of a DSA-Sepharose gel can be used effectively to discriminate malignant hCGs from non-malignant hCGs, obtained from urine samples. By assigning the cutoff value as 20%, desialylated hCG samples of patients with invasive mole and choriocarcinoma can be completely discriminated from those of patients with hydatidiform mole and pregnant women.

6. Functional role of the sialic acid residues of hCG

Many studies revealed that modification of the N-linked sugar chains of hCG alters its hormonal activity [35–38]. These reports indicate that complete removal of sialic acid residues from hCG enhances its binding to the target cells, but reduces its hormonal activity to 50%. Removal of the whole N-linked sugar chains from hCG further increases the binding of hCG to its target cells, but eliminates its hormonal activity almost completely. The deglycosylated hCG behaves as an antagonist to native hCG [39]. In an important article, Calvo and Ryan [40] reported that the glycopeptide mixture, obtained from hCG by exhaustive pronase digestion, blocks hCG signal transduction and suggested that a lectin like membrane component in addition to hCG-receptor may be involved in the signal transduction. Thotakura et al. reported that glycopeptides and oligosaccharides, obtained from other glycoproteins, can also prevent the hormonal action of hCG [41]. Matzuk et al. [42] investigated the bioactivities of hCGs, which lacked one of the four N-linked sugar chains. They prepared mutants of the hCG gene, in which one of the four *N*-glycosylation sites was eliminated by site-directed mutagenesis, and expressed them in Chinese hamster ovary cells. Only the hCG sample, which lacked the sugar chain at the Asn-52 of hCGα, significantly decreased in vitro signal transduction.

Expression of each subunit from the gene in CHO cells, after elimination of one of the two *N*-glycosylation sites by site-directed mutagenesis, revealed another role of the hCG N-linked sugar chains [43]. In hCGα, removal of Asn-78 glycosylation markedly reduced its assembly with hCGβ. Studies of hCGβ showed that the *N*-glycosylation is not essential for its assembly with hCGα, but elimination of Asn-30 glycosylation inhibits the secretion of uncombined hCGβ. These results indicated that the N-linked sugar chains of hCG are important for constructing the correct conformation of each subunit.

Goverman et al. [44] reported that deglycosylation of hCG by digestion with an exo- and endoglycosidase mixture from *D. pneumoniae*, which leaves the proximal *N*-acetylglucosamine residues of the four N-linked sugar chains of hCG intact, does not produce any dramatic effects on the folding and assembly of the two subunits. Therefore, the presence of at

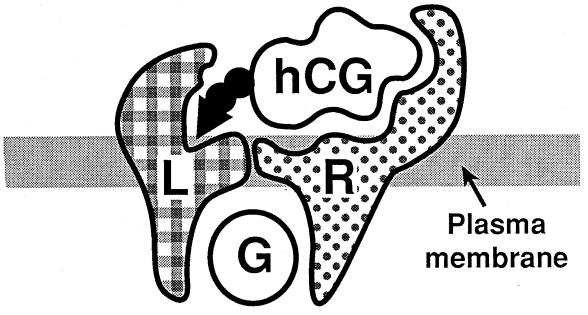

Fig. 6. Schematic presentation of hCG-receptor complex. G, Gs protein; L, lectin; R, hCG-receptor. Black circles and a triangle indicate the sugars. Taken from [49].

least one *N*-acetylglucosamine residue attached at each of the four *N*-glycosylation sites are enough to keep the correct folding of the two subunits. The role of N-linked sugar chains in intracellular folding of hCGβ was recently investigated by Feng et al. [45].

In order to elucidate the mechanism of suppression of hCG hormonal activity by desialylation, Amano et al. [46] investigated the functional role of the sialic acid residues of hCG. It was found that all sialic acid residues of the N-linked sugar chains of hCG occur as the Neu5Acα2-3Gal group [3]. To find out whether this particular group is included in the functional role of the sialic acid residues, the following experiment was performed.

MA-10 cells, a mouse Leydig tumor cell line established by Dr. Ascoli [47], produce cAMP in response to the addition of hCG in their culture medium. When hCG was desialylated, its hormonal activity was reduced to approx. 50%, as in the case of the other target cell lines. When the desialylated hCG was resialylated by incubation with CMP-Neu5Ac and Galβ1-4GlcNAc:α2-6-sialyltransferase, the isomeric hCG containing the Neu5Acα2-6Gal group gave almost the same dose-response curve as the natural hCG [46]. These results indicated that the sialic acid residues of hCG are important for the full expression of their hormonal activity in vitro, but the effect is independent of their linkage to the galactose residues.

Nemansky et al. [48] confirmed this finding and provided more important evidence. They found that the decrease in hormonal activity caused by desialylation of hCG was restored only by the addition of a Siaα2-6 residue, but not a Galα1-3 residue, to the galactose moiety of the Galβ1-4GlcNAcβ1-2Manα1-3Man arm of the N-linked sugar chains. They also found that further α6-sialylation of the Galβ1-4GlcNAcβ1-2Manα1-6Man arm reduced the hormonal activity of hCG, which indicates that the sialylation of the outer chain on the Manα1-3 arm, rather than the Manα1-6 arm, of the N-linked sugar chains of hCG plays an essential role in signal transduction. They also indicated that sialylation of the O-linked sugar chains is not important.

In order to elucidate the role of sialic acid residues in hCG signal transduction, an *N*-acetylneuraminic acid hexamer, isolated by partial sialidase digestion of colominic acid, was added to the reaction mixture of hCG and MA-10 cells. The oligosaccharide did not inhibit the binding of hCG to the surface of the target cells, but cAMP production was reduced to 50%, when 2 mM solution of the hexasaccharide was added [49].

These results indicate that the hexasaccharide can only inhibit the interaction of the sialic acid residues of hCG with the specific binding site on the cell surface, but did not influence the binding of the peptide portion of hCG to the receptor. The possibility that the action of the sialic acid hexamer may be due to a non-specific anionic polymer effect was refuted because the addition of fucoidin did not show any inhibition of the [^{3}H]hCG binding to the cell surface receptor or cAMP production by hCG.

The presence of the sialic acid binding site on the cell was confirmed using 3′-sialyllactose-conjugated BSA as a probe [49]. Based on data indicating that sialic acid residues bind directly to the cell surface, a model of the hCG-receptor complex was constructed and is shown in Fig. 6. A dual interaction of the peptide portion and the sialylated sugar chain of hCG with respective binding sites is essential for the signal transduction. However, it is not clear whether or not the lectin-like membrane component is a part of the hormone receptor or a part of a different molecule as shown in Fig. 6. In connection with this, it is of interest that a region homologous to the soybean lectin was found in the human hCG-receptor [50].

As already discussed, hCG contains four N-linked sugar chains. Amano and Kobata [8] studied which of the sugar chains interact with the lectin-like component on MA-10 cells. By investigating which sugar chains become resistant to sialidase digestion in the presence of MA-10 cells, they found that the sialic acid residues linked to N5 and N8 in Fig. 2 became resistant to sialidase digestion by binding to the target cells. However, the susceptibility of those linked to N6 was not affected by the interaction of the hormone with the target cells. These results indicate that the sialic acid residues of the sugar chains linked to the Asn-52 of hCGα and the Asn-30 of hCGβ were covered when hCG bound to the target cells. Consequently, it was concluded that one or both of the sugar chains at these two sites should play a critical role in signal transduction in the hormonal action of hCG.

7. Controversies

There are several reports which present interpretations that differ from those described in this review.

As introduced previously, Weisshaar et al. [7] confirmed the finding that site-specific distribution of the sugar chains occurs at the four *N*-glycosylation sites of hCG. However, they also found a wider distribution than that supposed by Mizuochi and Kobata 18 years ago based on the data of the sugar pattern analyses of commercial hCGα and hCGβ, which were guaranteed more than 99% pure [6]. Furthermore, they reported that small amounts of sialylated N9 and N10 in Fig. 2 are included as the sugar chain linked at Asn-52 of hCGα [7]. As shown in Fig. 1A, there are small peaks which migrated faster than peak III. These peaks, which Mizuochi and Kobata neglected to analyze, may contain these larger hybrid-type sugar chains.

Several papers have reported the presence of triantennary sugar chains in normal hCG samples purified from the urine of pregnant women. However, these samples were purified from the urine samples collected from many pregnant women. Since triantennary sugar chains occur in ample amounts on the urinary hCGs from choriocarcinoma patients, it cannot be ruled out that the larger sugar chains may have originated from choriocarcinoma hCGs contaminating the pooled urine. Our analyses of hCG samples purified from the urine of several healthy pregnant women always gave the pattern as shown in Fig. 1A. Absence of the triantennary sugar chains is evident from the elution pattern. Furthermore, the behavior of desialylated hCG samples from the urine of healthy pregnant women in a DSA-Sepharose column in Fig. 5 indicates that no triantennary sugar chains are included in these samples.

A paper, which could not be overlooked in this regard, was published by Elliott et al. [51]. By analyzing the carbohydrate structures of hCG samples, purified from urine of individuals with normal pregnancy, they also confirmed the occurrence of subunit-specific *N*-glycosylation, indicating that hCGα from normal pregnancy hCG contains N6 and N8 in Fig. 2 in the percent molar ratios of 49.3 and 36.7, and normal pregnancy hCGβ contains N5 and N6 in Fig. 2 as the major sugar chains. However, they also reported that hCGα contains small amounts of N1 and N5. Since they analyzed individual samples, the problem of choriocarcinoma hCG contamination can be ruled out. Using lectin affinity column chromatography, Skarulis et al. [52] found that glycosylation pattern of hCG changes as gestation progresses. Therefore, detailed structural analysis of the N-linked sugar chains of hCG produced at different times in the gestation period must be performed to solve this problem.

The crystal structure of hCG was reported by Lapthorn et al. [53]. It was indicated that only the sugar chain at Asn-52 of hCGα is present at the interface of the α- and β-subunits. The other three N-linked sugar chains are located on the outer face of the protein molecule. Purohit et al. [54] purified hCG samples from the culture media of insect cells transfected with the hCGα gene, lacking the potential *N*-glycosylation at Asn-52 or Asn-78, together with the intact hCGβ gene. By studies of the circular dichroism measurements and dissociation rates of the two mutant hCGs, they concluded that the absence of carbohydrate at Asn-52, but not at Asn-78, resulted in conformational changes in the mutant. Based on this evidence, they considered that the loss of hormonal activity of hCG with the absence of the sugar chain at Asn-52 was probably due to a conformational change in the heterodimer rather than to the loss of interaction of the Asn-52 sugar chain with the lectin on the target cells.

However, the conformational study of the sugar chains of hCG in solution by NMR revealed that the sugar chains at Asn-52 appear to extend into solution [55,56]. These data support the dual receptor theory. Quite recently, Thijssen-van Zuylen et al. [57] reported that the Asn-52-linked sugar chain of the hCG is not susceptible to digestion with peptide *N*-glycosidase F, in contrast to that of free hCGα. Therefore, more information is required for discussing the actual conformation of the sugar chain of hCG in solution.

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