Human chorionic gonadotropin: Different glycoforms and biological activity depending on its source of production

L’hormone chorionique gonadotrope humaine : différentes glycoformes et activités biologiques en fonction de sa source de production

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Abstract

Human chorionic gonadotropin (hCG) is the first hormonal message from the placenta to the mother. It is detectable in maternal blood two days after implantation and behaves like a super LH agonist stimulating progesterone secretion by the corpus luteum. In addition to maintaining the production of progesterone until the placenta itself produces it, hCG also has a role in myometrial quiescence and local immune tolerance. Specific to humans, hCG is a complex glycoprotein composed of two highly glycosylated subunits. The α-subunit is identical to the pituitary gonadotropin hormones (LH, FSH, TSH), contains two N-glycosylation sites, and is encoded by a single gene (CGA). By contrast, the β-subunits are distinct for each hormones and confer both receptor and biological specificity, although LH and hCG bind to the same receptor (LH/CG-R).

The hCG β-subunit is encoded by a cluster of genes (CGB) and contains two sites of N-glycosylation and four sites of O-glycosylation. The hCG glycosylation state varies with the stage of pregnancy, its source of production and in the pathology. It is well established that hCG is mainly secreted into maternal blood, where it peaks at 8–10 weeks of gestation (WG), by the syncytiotrophoblast (ST), which represents the endocrine tissue of the human placenta. The invasive extravillous trophoblast (iEVT) also secretes hCG, and in particular hyperglycosylated forms of hCG (hCG-H) also produced by choriocarcinoma cells. In maternal blood, hCG-H is elevated during early first trimester corresponding to the trophoblastic cell invasion process and then decreases. In addition to its endocrine role, hCG has autocrine and paracrine roles. It promotes formation of the ST and angiogenesis through LH/CG-R but has no effect on trophoblast invasion in vitro. By contrast, hCG-H stimulates trophoblast invasion and angiogenesis by interacting with the TGFβ receptor in a LH/CG-R independent signalling pathway. hCG is largely used in antenatal screening and hCG-H might represent a serum marker of implantation and early trophoblast invasion. In conclusion, hCG is the major pregnancy glycoprotein hormone, whose maternal concentration and glycan structure change all along pregnancy. Depending on its source of production, glycoforms of hCG display different biological activities and functions that are essential for pregnancy outcome.

Keywords: Glycoproteins; Glycosylation; Structure-function; Receptors; Syncytiotrophoblast; Extravillous trophoblast; Invasion

Résumé

L’hormone chorionique gonadotrope humaine (hCG) est le premier message hormonal produit par le placenta vers l’organisme maternel. L’hCG est détectable dans le sang maternel, deux jours après l’implantation et se comporte comme un super agoniste de la LH stimulant la sécrétion de progestérone par le corps jaune. En plus de maintenir la production de la progestérone jusqu’à ce que le placenta en produise, l’hCG intervient dans la quiescence du myomètre et la tolérance immunitaire locale. Spécifique à l’espèce humaine, l’hCG est une glycoprotéine complexe composée de deux sous-unités fortement glycosylées. La sous-unité α est identique aux hormones gonadotrophines hypophysaires (FSH, LH, TSH). Elle est codée par un gène unique (CGA) et contient deux sites de N-glycosylation. En revanche, les sous-unités β sont distinctes pour chacune des hormones et confèrent la spécificité biologique en se liant à leur récepteur respectif bien que LH et hCG reconnaissent le même récepteur (LH/CG-R). La sous-unité β de l’hCG est codée par un cluster de gènes (CGB) et contient deux sites de N-glycosylation et quatre sites de O-glycosylation. La glycosylation de l’hCG, dépend du stade de la grossesse, de sa source de production et de la pathologie. Il est bien établi que l’hCG est principalement sécrétée par le syncytiotrophoblaste (ST), qui représente le tissu endocrine et d’échanges du placenta humain. Sa concentration dans le sang maternel...
carnalisation from 8–10 weeks of gestation (WG). The trophoblast extravillous (iEVT) secretes specifically fHCG (hCG-H) which are also produced by the cells of choriocarcinoma. In the maternal circulation, hCG-H is elevated at the beginning of the third trimester corresponding to the physiological invasion trophoblastic. Out of its endocrine role, hCG-H could represent a marker specific of the implantation and of the invasion trophoblastic. In conclusion, hCG is the hormone glycoprotétique majeure of the gissesse, which the concentration materno-serique and the glycosylation evolve over the entire pregnancy. In function of its production, the glycoforms of hCG present different activities biologiques et des fonctions différentes qui sont essentielles à l’issue de grossesse.

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Mots clés : Glycoprotéine ; Glycosylation ; Structure-fonction ; Recepteur ; Syncytiotrophoblaste ; Trophoblaste extravilleux ; Invasion

1. Human placenta development

The placenta is a transitory organ necessary for pregnancy and foetal growth. The human placenta is characterized by extensive invasion of the uterus wall by the trophoblast, allowing direct contact of the trophoblasts with maternal blood (hemochorial placenta), by considerable changes in the vasculature of the uterus and by the extent and specificity of its hormonal production [1]. The chorionic villus represents the structural and functional unit of the human placenta and is bathed in maternal blood within the intervillous space from the end of the first trimester of pregnancy when trophoblast plugs are removed and the uterine spiral arteries (usa) remodelled. After the initial phase of nidation, human cytotrophoblasts differentiate along either the villous or extravillous cytotrophoblast pathway (Fig. 1A and B) [2].

Extravillous cytotrophoblasts (EVT) are located at the tip of the anchoring chorionic villi contacting the uterine wall. They are involved in anchoring the placenta into the uterus and participate to immunotolerance and remodelling of uteroplacental vascularization. EVT proliferate to form multilayered columns of cells and then exit the cell cycle and invade the decidua up to the upper third of the myometrium. EVT also specifically migrate toward and invade the usa through endovascular and perivascular routes replacing the endothelial lining and most of the musculoelastic tissue, leading to low-resistance vessels that escape to vasoconstrictor mechanisms. This invasion process and remodeling of the uterine arteries are essential to ensure an adequate supply of maternal blood necessary for foetal growth [1–4]. It is finely regulated during pregnancy (first trimester) and space (oriented towards the usa). Shallow trophoblast invasion and defective usa remodelling during first trimester are often associated with foetal growth restriction (FGR) and preeclampsia. Preeclampsia is a major and frequent complication of human pregnancy (about 3–7% corresponding to more than 16,000 pregnancies per year in France), with serious maternal and foetal (FGR) consequences. It has been identified as one of the first causes of severe prematurity. To date, there is neither curative nor preventive treatment for preeclampsia, except delivery of the placenta.

The mononucleated villous cytotrophoblasts (VCT) form an epithelium that covers the floating chorionic villi containing the foetal-placental vessels. VCT aggregate and fuse with the outerlayer – a multinucleated syncytiotrophoblast (ST) – which is renewed all along pregnancy and in direct contact with maternal blood within the intervillous space. This ST ensures exchanges of gases and nutrients between maternal and foetal blood. The ST represents also the endocrine tissue of the placenta, secrete large amounts of hormones, including human chorionic gonadotropin (hCG) [5]. Thus, anomalies in ST formation or function may interfere with main functions of the placenta and directly alter foetal growth.

The deep invasion of the EVT within the uterine wall, remodelling of usa and the important ST hormonal functions (in particular hCG), are specific to humans. Poor placentaion is directly involved in many pregnancy diseases, including FGR, preeclampsia and prematurity.

2. Human chorionic gonadotropin

Specific to humans, hCG is a complex and highly glycosylated glycoprotein of about 37 kDa composed of two glycosylated subunits which are non-covalently associated. The α-subunit is identical to the pituitary gonadotropin hormones (luteinizing hormone: LH, follicle-stimulating hormone: FSH, thyroid-stimulating hormone: TSH), contains 92 amino acids with two N-glycosylation sites, and is encoded by a single gene (CGA) located on chromosome 6q21.1-23 [6]. The β-subunits are distinct and confers the biological specificity to the hormone. The hCG β-subunit contains 145 amino acids with two sites of N-glycosylation and four sites of O-glycosylation, and is encoded by a cluster of genes that have evolved by duplication from LHB. The CGB subunit is encoded by any one of the six nonallelic genes CGB8, CGB7, CGB5, CGB3, CGB2, and CGB1 present on chromosome 19q13.32 [7–9]. CGB1 and CGB2 are two pseudogenes, whereas the other CGB genes are regrouped in two subtypes: type 1 (CGB7) and type 2 (CGB3, CGB5, and CGB8), which correspond to two different proteins that differ from three amino acids [10]. CGB5 is expressed predominantly in the placenta [11]. Whereas hCGα is produced in large excess, hCGβ represents a step limiting for hCG synthesis.

After implantation, hCG is the first trophoblast signal detected in the maternal blood and is used to diagnose pregnancy. hCG and free hCGβ are detected in the maternal blood from the first week of gestation (WG), and their levels increase until reaching a peak at 10–12 WG and then decrease gradually.
whereas hCGα levels increase progressively up to term [12]. Maintenance of pregnancy during the first trimester depends on the synthesis of hCG, which prevents regression of the corpus luteum [13] allowing the maintenance of ovarian progesterone secretion [5]. In addition to its well-established endocrine role, hCG has a role in promoting angiogenesis in the uterine endothelium [14], in maintaining the quiescence of the myometrium [15], and in contributing to maternal immunotolerance [16]. HCG also promotes formation of the syncytiotrophoblast in an autocrine manner through binding to the LH/CG-R [17]. Binding of hCG to its receptor present at the surface of mononucleated villous cytotrophoblast activates adenylate cyclase, phospholipase C and ion channels, which in turn control cellular cAMP, inositol phosphates, Ca^{2+} and other second messengers [18]. Cyclic AMP induces trophoblastic cell fusion [19] and increases mRNA and protein levels of the fusogenic protein syncytin-I in vitro [20].

HCG is highly glycosylated since more than 30% of its structure is constituted by carbohydrate residue. The sugar branches covalently bound to the peptide chains consist of O-linked oligosaccharide containing an N-acetylgalactosamine residue linked to either a serine residue and N-linked oligosaccharide containing an N-acetylglucosamine residue linked to an asparagine residue. The alpha subunit contains 2 N-glycosylation sites and the beta subunit 2 N and 4 O-glycosylation sites located in the carboxyl terminus [21]. Secretion, biological activity and half-life of hCG depend on the glycosylated state of the hormone (micro heterogeneity due to the variability of oligosaccharide moiety). The sialic acid content of hCG has a major significance in isoelectric point.
(PI), its receptor binding ability, biological activity and clearance from the maternal circulation [22]. It is important to note that there is not only one hCG but a family of glycoforms that can be visualized by 2D-electrophoresis (separation in function of both PI and molecular weight) following by immunoblotting using a polyclonal anti-hCG\(\beta\) antibody able to recognize several glycoforms (Fig. 2A).

A hyperglycosylated form of hCG (hCG-H) – the so-called invasive trophoblast antigen (ITA) – has been characterized from urine of a patient with choriocarcinoma [23]. An antibody (B152) was raised against the C-terminus O-linked oligosaccharides of the \(\beta\)-subunit [24]. Hyperglycosylated hCG (hCG-H) is a family of glycoproteins with the same polypeptide structure as hCG, but with larger N- and O-linked oligosaccharides. The oligosaccharides increase the molecular weight of hCG from 36,000–38,000 kDa to 40,000–43,000 kDa, depending on the extent of hyperglycosylation (Fig. 2B). hCG-H has triantennary N-linked oligosaccharides and double molecular size O-linked oligosaccharides (hexasaccharide compared with predominantly trisaccharide structures) [25].

Abnormal glycosylated hCG has been reported in the case of T21-affected villous trophoblasts. It presents a lower bioactivity compared to hCG produced by normal cells in term of LH/CG-R signalling [26]. This weakly bioactive hCG from T21-placenta is involved in the defect of syncytiotrophoblast formation in vitro [27].

3. Comparative study between hCGs from villous versus extravillous trophoblasts

The placenta is the main source of hCG during pregnancy. In addition to their expression by trophoblastic cells during pregnancy, hCG\(\beta\) and hCG are slightly produced by normal tissues and expressed in gonadal and non gonadal neoplasms [28,29]. In human placenta it is well established that the syncytiotrophoblast (ST) is the main source of hCG production and secretion. Ninety-nine percent of the hCG produced during pregnancy is secreted in maternal blood by the ST. Interestingly, we recently reported in situ and in vitro that villous trophoblasts from early first trimester (8–9 WG, before the entrance of maternal blood into the intervillous space due to the presence of trophoblastic plugs) express more hCG (transcripts and protein levels) than trophoblasts from later gestation when the chorionic villi are perfused with maternal blood raising the oxygen levels from 2–3 to 8% (12–14 WG) [30]. This observation might explain in part the maternal plasmatic peak of hCG during the first trimester of pregnancy.

In addition to the ST, it has been shown in situ and in vitro that non proliferative human invasive extravillous trophoblasts (iEVT that are negative for the cell cycle marker Ki67) also express and secrete hCG, suggesting an autocrine/paracrine function at the maternal-foetal interface for this hCG from EVT origin. This hCG glycoforms were found to be more acidic and of higher molecular weight as shown by 2D-electrophoresis analysis (Fig. 2C) [31,32].

Using the B152 antibody, hyperglycosylated forms of hCG (hCG-H, mainly produced by choriocarcinoma JEG-3 cells) was quantified in each trophoblast subtype cell supernatants i.e. ST and iEVT. The authors found that hCG-H represent about 20% of total hCG secreted in vitro by iEVT isolated from 9 WG-chorionic villi, whereas it was almost undetectable in culture medium from ST obtained from the same placentas. Interestingly, only iEVT conditionned medium containing hCG promotes trophoblast invasion in vitro (ten time increase for 1 nM hCG from iEVT cultures); hCG secreted in vitro by the ST has no effect compared to controls [32,33].

Since hCG secreted by the ST does not affect trophoblast invasion, it is unlikely that hCG from iEVT origin...
signals through LH/CG-R dependent signalling pathway. Others reported that hCG modulated the invasion process in a LH/CG-R independent manner [34–36].

These results offer strong evidence that hCG glycoforms secreted in vitro by iEVT, likely hCG-H, but not by the ST (only hCG), participate to the control of the trophoblast invasion process in an autocrine manner through a LH/CG-R independent pathway. In situ, hCG-H was immunolocalized specifically in invasive and endovascular EVT from 9 WG placenta tissue sections, but not in the syncytiotrophoblast. Endovascular EVT being in direct contact with maternal blood within the lumen of usa, hCG-H was measured in maternal blood. Quantification of both hCG and hCG-H was performed in more than 500 maternal sera collected between 9 and 19 WG during normal pregnancies. By contrast to hCG that picks at about 11 WG and then decreases as previously reported by numerous studies, hCG-H levels are high at 9 WG and continuously decrease during pregnancy reaching a plateau at the beginning of the second trimester corresponding to the end of the trophoblastic cell invasion process [33]. Recently, we provided evidence that like hCG, hCG-H displayed a potent angiogenic effect. However, using LH/CG-R KO mice we demonstrated that hCG-H induces angiogenesis independently of LH/CG-R signalling. Indeed, using coimmunoprecipitation, competitive binding, TGFβ reporter gene assays, inhibitors and Smad immunoblotting, we showed that hCG-H interacts with the TGFβ signalling pathway for its angiogenic effect [37].

Structural data indicate that the additional sugar chains prevent a complete folding of the heterodimer, allowing the unmasking of the cryptic central cystine knot domain. Such structures have been identified in a number of factors that form the cystine knot growth factor family including TGFβ. The structural similarities between hCG-H and TGFβ suggested that the exposed cystine knot structure in hCG-H might interact with the TGFβ receptor, resulting in decreased first-trimester trophoblast apoptosis and enhanced invasion associated with secretion of metalloproteinases. All together, these results suggest that the high levels of hCG-H observed in first trimester maternal sera are mainly from endovascular EVT origin, reflecting the early trophoblast invasion process. In early first trimester hCG-H is produced by the invasive and endovascular EVT that signal through TGFβ, whereas hCG is mainly produced by the ST in large amount at about 10 WG and signal through the LH/CG-R.

Thanks to our human trophoblast primary cell culture models, we were the first to describe different glycoforms, signalling and functions for hCGs depending on its source of production i.e. endocrine syncytiotrophoblast (ST) or invasive extravillous trophoblast (iEVT) (Fig. 3). But, little is known about the structure and activity of all the hCG glycoforms produced by the trophoblast subtypes from the implantation to the end of the

Fig. 3. hCGs: different sources for different functions and signalling pathway [31–33,37].
invasion process (end of first trimester). Indeed, hCG biochemical characterization was based on urine and sometimes serum from pregnant women and its biological role studied using these samples or recombinant hCG. Because hCG is highly metabolized in serum and urine giving multiple forms of cleaved and degraded hormone (nicked hCG, nicked βhCG, β-core...) it does not reflect native hCG produced by the trophoblast. hCG is a complex glycoprotein hormone whose bioactivity and then functions are highly dependent on the composition of its carbohydrate moiety and therefore from its source of production and term of pregnancy.

As described above hCG-H may present a good serum marker of early physiological EVT trophoblast invasion to screen pregnancy diseases from placental origin [33]. hCG-H was measured in urine of pregnant women on the first day of hCG detection (about one week after conception, hCG > 1 mU/mL). In all term pregnancies, the proportions of hCG-H/hCG on the day of implantation were greater than 50%. Statistically significant lower ratio of hCG-H/hCG less than 50% were observed in more than half of spontaneous abortions [38,39], hCG-H is also reported as an early predictor of pregnancy outcomes after in vitro fertilization [40,41].

The potential usefulness of these circulating glycoforms in the maternal sera for the diagnosis of pathological pregnancy from placental origin such as preeclampsia was recently tested. Pathological pregnancies (about 70,000 per year in France) have both short-term and long-term consequences for the mother (death, metabolic disorders, etc.), the baby (death, growth restriction, metabolic disorders in adulthood) and major costs for society. Many complications of pregnancy and parturition are related to placental or uteroplacental interface dysfunction. Therefore, production of new markers from placental origin to improve the early diagnostic of pregnancy or implantation pathologies represents a major challenge.

Recently, two studies reported that low concentrations of hCG-H in first trimester (8–13 WG) maternal sera are associated with subsequent preeclampsia especially its early onset form [42]. The same team showed that in second trimester (14–17 WG) hCG-H in maternal serum does not predict preeclampsia [43]. This is consistent with our results suggesting that hCG-H is an early marker (first trimester) of physiological trophoblast invasion [33].

4. Conclusion

hCG is the major pregnancy glycoprotein hormone, whose maternal concentration and glycan structure change all along pregnancy. Depending on its source of production, glycoforms of hCG display different biological activities and functions that may play a role in determining pregnancy outcome.

Disclosure of interest

The authors declare that they have no competing interest.

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