Human Chorionic Gonadotropin

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Human chorionic gonadotropin is a glycoprotein hormone produced by the trophoblast during pregnancy as well as by both trophoblastic and non-trophoblastic tumors.

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1. Introduction

The name human chorionic gonadotropin (CG) has been first coined in 1920, when Hirose showed a relationship between the hormone synthesized by the human placenta and the production of progesterone by the corpus luteum cells [1]. The structural model of hCG protein was developed and presented in the 1970s. It shows that human chorionic gonadotropin is a heterodimeric hormone belonging to the group of glycoproteins (GPH). Other hormones, which can be distinguished in this group are: luteotropin (LH), thyrotropin (TSH) and follicle stimulating hormone (FSH) [2]. All these hormones, called sex glycoproteins, are synthesized by the pituitary gland, while hCG is formed in the placenta and is mainly produced by syncytiotrophoblast cells during pregnancy [3].

hCG plays a key role throughout the development of pregnancy. Under physiological conditions hCG participates in and regulates many physiological processes which include: maintenance of progesterone production by the corpus luteum, chorionic villi development, embryo implantation, angiogenesis and mother's immunotolerance to the developing fetus [4][5][6].

Synthesis of hCG starts in the blastocyst after the hatching, however beta subunit of the hormone presence was reported at the 2PN (2 pronuclear) stage in embryos. The highest levels of hCG in the blood serum and urine are observed ten weeks after fertilization. Afterwards the hormone concentration decreases, but a relatively high level is sustained until the end of pregnancy [7][8].

2. hCG Measurement

By measuring hCG concentration in blood or urine, it is possible to reliably diagnose pregnancy and determine its stage. Serum level of hCG is also used to monitor pregnancy and pregnancy disorders. Low levels of the hormone compared to a normal pregnancy is noted primarily in ectopic pregnancy and cases of spontaneous abortions. It can also be an indicator of failure of the *in vitro* fertilization procedure. Low hCG levels in the second trimester may also be associated with chromosome 18 trisomy of the fetus. On the other hand, a significantly increased level of chorionic gonadotropin during pregnancy may indicate chromosome 21 trisomy of the child Elevated hCG levels are characteristic of molar pregnancy as well as trophoblast hyperplasia and neoplasms [8][7][9].

3. hCG Structure

hCG is a heterodimeric glycoprotein hormone, which comprises two non-covalently linked subunits: alpha (α) and beta (β). The highly conserved α subunit is common to all gonadotropic hormones, such as follicle stimulating hormone (FSH), thyrotropin (TSH) and luteinizing hormone (LH), while the β subunit is hormone specific and determines its biological properties [2][3][10]. Since placental hCGα is always produced in excess, the factor limiting the total amount of secreted hCG seems to be the expression level of the beta subunit [11].

The alpha and beta subunits of hCG are encoded by distinct genes that are found on different chromosomes. The 92-amino acid alpha subunit (P01215, UniProtKB / Swiss-Prot) is encoded by a single *CGA* gene located on chromosome 6 (6q14-q21, NC_000006.11), while the 150-amino acid beta subunit (P01233, UniProtKB / Swiss-Prot)) is encoded by six allelic genes (*CGB3*, *CGB5*, *CGB6*, *CGB7*, *CGB8*, *CGB9*) located in the LHB/CGB cluster on chromosome 19 (19q13.32, NC_000019.9). Despite the high homology of their regulatory elements, the expression level of individual *CGBs* is uneven

[12][13][14]. The genes reported to be the most transcriptionally active in pregnancy are *CGB3* and its allele *CGB9,* as well as *CGB8* and especially *CGB5*. In fact *CGB5* is the gene, which was most extensively studied in the context of hCGβ expression regulation. *CGB1* and *CGB2* genes are less transcriptionally active and their protein products have never been identified. What is more *in silico* predictions show that CGB1 and CGB2 polypeptides, which are 132 amino acid long, are not homologous to consensus hCGβ protein [15][16][17][18][19][20][21].

4. hCG Expression Regulation

The mechanisms responsible for the regulation of chorionic gonadotropin expression have not been fully investigated. Separate promoter regions of the genes encoding the alpha subunit and the beta subunit are controlled by different groups of transcription factors, and the classic TATA box present in the promoter of the *CGA* gene has not been identified in the promoter sequence of any of the allelic *CGB* genes [22][23].

The *CGA* promoter region includes two copies of the cAMP-responsive element (CRE) and thus *CGA* expression is depends on transcription factors such as CREM, CREB-1 and ATF-1 [22][24][25].

Understanding the mechanisms responsible for the regulation of the expression of the beta subunit is relatively difficult due to the high homology of the genes encoding hCGβ [26]. The GC-rich promoter region is the binding site of the following transcription factors: SP1, SP3, AP2, OCT4, and C-JUN [23][27][28][29][30]. Another important factor influencing the transcription process of *CGB*s are epigenetic changes occurring in the regulatory sequences. Variations in methylation status of the *CGB* promoters in chorionic and placental tissue as well as in ovarian cancer have been previously shown [17][30][31][32][33].

As individual genes encoding the beta hCG subunit differ in single nucleotides translation of hCG yields two forms of the protein differing in one amino acid, which is either alanine or aspartic acid in position 117. This difference seems however not to have any functional significance [15].The native form of human chorionic gonadotropin hormone consists of two subunits alpha and beta.

The alpha subunit with a molecular weight of 14.9 kDa consists of 92 amino acids that form eight beta chains and one alpha helix. The molecule is stabilized by five disulfide bridges formed between cysteines of hCGα [34].

The beta subunit consists of 145 amino acids. In its structure, there are seven beta chains that are linked, similarly to the alpha subunit, by disulfide bridges. The molecular weight of hCGβ is 23.5 kDa. The amino acid sequence of this subunit shows eighty percent homology to the β chain of the LH hormone. The differences between the chains of these proteins are due to the carboxyl terminus being 24 amino acids longer in the case of the beta subunit of hCG. The presence of these extra amino acids increases the gonadotrophin half-life compared to the luteotropic hormone, where they are absent [9][35].

The beta subunit can form a dimer with hCGα, exist as a free β subunit; it also has the ability to form homodimers (ββ). The presence of homodimers is noticeable primarily in neoplasms.

In the structure of the chorionic gonadotropin, the presence of a cysteine knot can be distinguished [35]. This motif is characteristic for growth factors such as: TGFB (transforming growth factor beta), PDGFB (platelet-derived growth factor) and NGF (nerve growth factor). They are all responsible for the regulation of cell proliferation [35][36]. Homology in the structure of these factors in relation to the chorionic gonadotropin seems to explain its influence on cells by regulating their proliferation [37].

Seventy percent of the hormone's weight are protein chains, while the remaining thirty are carbohydrates added to the protein as a form of posttranslational modifications [2]. Sugar residues linked by an N-glycosidic bond to asparagines are present in both hCGα and hCGβ. However the beta subunit is characterized also by sugars attached via the O-glycosidic bond with serines [7][38][39][40]. The degree of glycosylation of hCG can vary in terms of the number and type of sugar residues added. The attached oligosaccharides can be in the form of straight or branched molecules. This leads to the formation of hormone variants that differ in molecular weight. The basic variant of CG has a mass of about 36.7 kDa, but it has been observed that trophoblast cells can also secrete hCG molecules with a mass of 38.5–40 kDa [8]. hCG variants with an increased content of sugar and thus also an increased molecular weight are termed hyperglycosylated chorionic gonadotropin (H-hCG). It has been suggested that these forms perform different but complementary biological functions [7].

Folding of the hCG subunits, assembly of the holohormone as well as initial post-translational modifications take place in the endoplasmic reticulum (ER) [41]. During the next steps the hormone leaves ER and is carried to the Golgi apparatus for further modifications. The complete hormone is then packed in vesicles and undergoes exocytosis [42].

5. hCG Mechanisms of Action

Under physiological conditions, hCG acts via the membrane-anchored LHCGR receptor. The heterodimeric hCG molecule activates the receptor which results in a signaling cascade leading to an increase in the activity of adenylate cyclase and the synthesis of cAMP in the cell. When the signal reaches the cell nucleus, the transcription of selected genes is activated [43].

However, such a mechanism is only possible with heterodimers and provided that the cells express the LHCGR receptor. Meanwhile, hCG is also produced by tumors and not all tumors express the LHCGR receptor [44][45].

An alternative mode of hCG action is based on the similarity of hCG to growth factors having the cysteine knot motif. In fact, it has already been demonstrated that free hormone subunit acts competitively with TGFβ and increases the population of cancer cells, exerting an anti-apoptotic effect [39]. Recently, this hypothesis was additionally verified in studies which showed that hyperglycosylated human chorionic gonadotropin supports trophoblast invasion and angiogenesis through TGFβ receptor activation [46].

It has been also documented that in Leydig, granulosa-luteal and theca interstitial cells hCG may exert its biological effects by the activation of extracellular signal-regulated kinase (ERK) and protein kinase B (AKT) [47][48]. The same mechanism was confirmed to be present in cancer cells in which hCG stimulation increases ERK and AKT kinases phosphorylation, while these biological effects are independent of LHCGR [49].

6. hCG in Cancer

Apart from pregnancy, human chorionic gonadotropin, especially its β subunit (hCGβ) is also secreted in gestational trophoblastic disease, and by a large group of non-trophoblastic tumors.

hCGβ expression was confirmed in tumors of different origin such as: breast, cervix, prostate, lung, colon, kidney, bladder, pancreas, anus, vulva, ovary, brain, endometrium and mouth [50][51]. hCGβ appears to be a an agent driving tumor growth, cancer progression and metastasis. It also determines the response to treatment and survival of cancer patients [48][49][50]. Unfortunately the presence of hCGβ correlates with unfavorable prognosis of cancer patients.

Even though many studies document hCGβ impact on cancer cells biology, the mechanisms behind hCGβ action in cancer remain unexplained. Still there is strong evidence that hCG, and especially its beta subunit, are autocrine growth factors, which facilitates progression of a cell to metastatic cancer [9]. It has been postulated that as in pregnancy also during oncogenesis the hormone stimulates neovascularization and leads to the development of cancer-tolerance by the patient's immune system [5][52]. The hCG beta subunit has also been shown to have an anti-apoptotic effect on tumor cells, induce proliferation and tumor growth [53][54][55][56]. It has also been proven that hCGβ expression regulates the epithelial-mesenchymal transition (EMT). Specifically, hCGβ was shown to promote migration and invasion of ovarian, lung and colorectal cancer cells [57][58][59]. The expression of genes coding for hCGβ was confirmed in blood of patients with gynecological cancers but also head and neck, breast, digestive system, lung, prostate and pancreas tumors. This phenomenon might indicate the presence of circulating tumor cells (CTCs) [60].

Since hCG and hCGβ are tumor promoting factors there were attempts to block the molecule with an anti-hCG vaccination. A vaccine based on hCGβ showed clinical benefits in patients with colorectal cancer, who showed better survival rates than other patients [61].

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