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Review Article

HCG variants, the growth factors which drive human malignancies

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Abstract: The term human chorionic gonadotropin (hCG) refers to a group of 5 molecules, each sharing the common amino acid sequence but each differing in meric structure and carbohydrate side chain structure. The 5 molecules are each produced by separate cells and each having separate biological functions. hCG and sulfated hCG are hormones produced by placental syncytiotrophoblast cells and pituitary gonadotrope cells. Hyperglycosylated hCG is an autocrine produced by placental cytotrophoblast cells. Hyperglycosylated hCG drives malignancy in placental cancers, and in testicular and ovarian germ cell malignancies. hCGβ and hyperglycosylated hCGβ are autocrines produce by most advanced malignancies. These molecules, particularly the malignancy promoters are presented in this review on hCG and cancer. hCGβ and hyperglycosylated hCGβ are critical to the growth and invasion, or malignancy of most advanced cancers. In many ways, while hCG may appear like a nothing, a hormone associated with pregnancy, it is not, and may be at the center of cancer research.

Keywords: Human chorionic gonadotropin (hCG), variants, groth factors, human malignancies, cancer

Introduction

Human chorionic gonadotropin (hCG) is a glycoprotein hormone comprising an α-subunit and β -subunit (**Figure 1**). hCG is considered the most acidic and most glycosylated glycoprotein (**Table 1**). The sugars form a key part of hCG's structure. The structure of the sugars on hCG are shown in **Figure 2**. **Figure 1** shows the 3 dimensional structure as predicted from X-ray crystallography [2].

Interestingly, the β-subunit (hCGβ) has common evolutionary sequences with transforming growth factor β (TGFβ) [3, 4]. Examination of the crystal structure of hCG [2] shows the presence on hCGβ of a cystine knot structure also common to TGFβ and other cytokines. This site of this cystine knot structure is shown in **Figure 1**. It comprises 4 overlapping β-subunit peptides, β30-45, β80-100, β1-15 and β50-65 linked by 3 disulfide bridges, β34-88, β9-57 and β38-90. While the hormone hCG does not apparently expose these sequences and structures common to TGFβ, hCG variants can. As found, hyperglycosylated hCG, hCGβ and hyperglycosylated hCGβ all can seemingly antagonize a TGFβ receptor [5, 6]. As described later in this review, all these molecules are autocrine cancer promoters that seemingly act by antagonizing a TGFβ receptor on cancer cells.

Hyperglycosylated hCG is a second major form of hCG that seemingly functions as a TGFβ antagonist [6]. As such the amino acid sequence generates two independent dimeric molecules, hCG and hyperglycosylated hCG. While hCG functions as a hormone acting on the joint hCG/luteinizing hormone (LH) receptor, hyperglycosylated hCG functions as an autocrine as an apparent TGFβ antagonist and is produced by cytotrophoblast cells [6, 7]. hCG and hyperglycosylated hCG act together to control implantation of pregnancy and placental growth and function during pregnancy. Hyperglycosylated hCG is an over-glycosylated variant of hCG. As shown in **Table 1** and **Figure 2**, hyperglycosylated hCG has double size O-linked oligosaccharides and extra-large N-linked oligosaccharides. Considering the size of these oligosaccharides, they account for 39% of the molecular weight (**Table 1**).

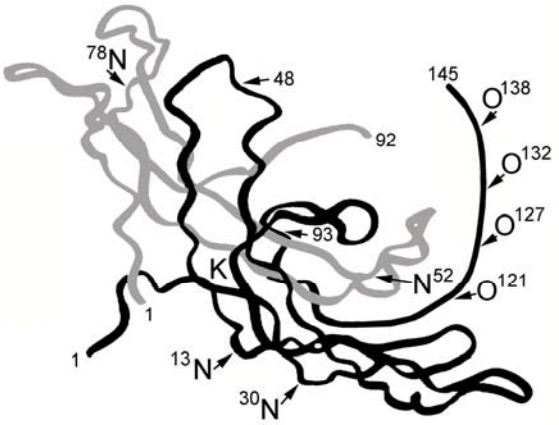

Figure 1. Crystal structure of deglycosylated regular hCG, as shown by Lathorn et al [2]. The unfolded ß-subunit C-terminal peptide is added (missing in crystal structure). It is inferred that this structure is not folded since the sequence comprises primarily a polymer of proline and serine residues. The symbols N and O indicate the sites of attachment of N-linked and O-linked oligosaccharides. Residue ß48 and ß93 are indicated as the site of cleavage of the ß-subunit. The symbol K indicates the site of the cystine knot structure. The α-subunit is shown in grey, and ß-subunit is shown in black.

Hyperglycosylated hCG is the principal molecule produced in the first 3 weeks of pregnancy. At this time is controls implantation of pregnancy, and cytotrophoblast cell growth and invasion during the first trimester of pregnancy [8-14]. It is our understanding that antagonization of the cytotrophoblast cell TGFβ receptor leads to a cancer-like process, blockage of apoptosis, and secretion of invasive enzymes, metalloproteinases and collagenases, leading to growth and proteolytic invasion [15-25].

Hyperglycosylated hCG function for the length of pregnancy promoting root cytotrophoblast cell growth. The combination of hCG and hyperglycosylated hCG promote villous placental tissue growth, hyperglycosylated hCG promoting cytotrophoblast growth and hCG promoting the fusion of cytotrophoblast cells to syncytiotrophoblast cells. hCG also promoted umbilical artery angiogenesis and formation of the umbilical circulation. All these system come together in formation of hemochorial placentation [26-34].

Table 1. Properties of 5 independent variants of hCG. Amino acid content, molecular weight and sugar contents determined from published structures as determined by Elliott et al. for hCG and hyperglycosylated hCG [90], Birken et al. for sulfated pituitary hCG [80] and Valmu et al. for hyperglycosylated hCGß [91]. The molecular weight of common hCG dimer amino acid backbone is that as determined by Morgan et al. [122]. Molecular weight of N- and O-linked sugar side chains is added to these values. Isoelectric points are those published by Sutton et al. [92], and metabolic clearance rates are those established [1, 80].

Parameter	hCG	Sulfated hCG	Hyperglycosylated hCG	hCGß	Hyperglycosylated hCGß
Type of molecule	Hormone	Hormone	Autocrine	Autocrine	Autocrine
Total Molecular weight	37,180	36,150	42,800	23,300	27,600
Amino acids α-subunit	92	92	92		-
Amino acids ß-subunit	145	145	145		145
Peptide molecular weight	26,200	26,200	26,200	16,000	16,000
O-linked sugar units	4	4	4	4	4
N-linked sugar units	4	4	4	2	2
Molecular weight sugars	10,980	9,950	16,600	7,300	11,600
Percentage sugars	30%	28%	39%	31%	42%
Isoelectric point (pI), principal peak	3.5	Not known	3.2	Not known	3.5
Metabolic clearance rate	36 h	20 h	Not known	0.72 h	Not Known

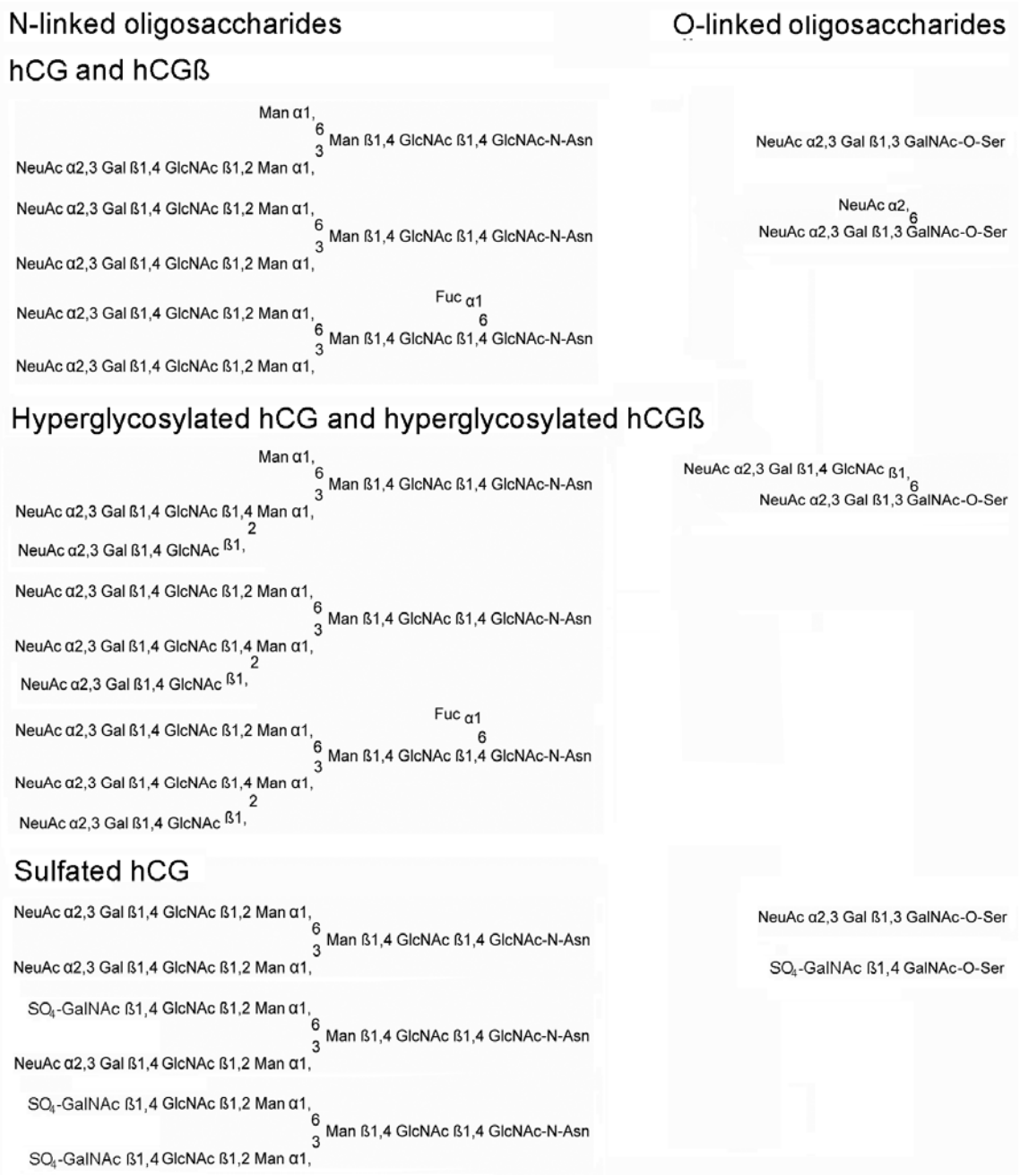

Figure 2. The carbohydrate structure of hCG, hCGß, hyperglycosylated hCG, hyperglycosylated hCGß and sulfated hCG [80,90,91].

hCG and hyperglycosylated hCG evolved with humans [35, 36]. During their evolution came super-CG (chorionic gonadotropin) and super-hyperglycosylated CG two extremely potent growth factors that permitted hemochorial placentation to extend its efficiency multiple-fold in humans [35, 36]. This was needed to permit the development of the human brain and humans [35, 36]. The human genome harbors genes to express super-hyperglycosylated CG and its derivatives, super-CGβ and super-hyperglycosylated CGβ. These are expressed in human can-

Table 2. Parallelisms between placental implantation and invasion characteristics in primates, presence and sugar structure on chorionic gonadotropin (CG) or LH, and relative brain masses. Table summarizes published data [37-40,49,55].

Species	Implantation characteristics	Depth of Invasion	Sugar structures, acidity or pI	Brain mass (% of body weight)	First appearance
Humans	Hemochorial	1/3rd myometrium	CG, 8 oligosaccharides, pI 3.5	2.4%	0.1 million year ago
Advanced simian primates	Hemochorial	1/10th myometrium	CG, 6 oligosaccharides, pI 4.9	0.74%	20 million year ago
Early simian primates	Hemochorial	through decidua	CG, 5 oligosaccharides, pI 6.3	0.17%	37 million year ago
Prosimian primate	Epitheliochorial	no-invasion	No CG produced, LH produced, 3 oligosaccharides, pI 8.4	0.07%	55 million years ago

Table 3. Use of serum free ß-subunit (hCGß plus hyperglycosylated hCGß) as a tumor marker for detection of malignancies. Averages are determined by combining total positive cases from multiple reports (89-79,100-113).

Malignancy	Number of Cases	Sensitivity (>3 fmol/ml)
Ovarian cancer	150	38%
Cervical cancer	60	37%
Endometrial cancer	55	33%
Vulvar	50	38%
Bladder cancer	170	35%
Lung cancer	243	18%
Colorectal cancer	436	17%
TOTAL	1164	Mean 30% detection

cers, and just as they permitted super-biology in human evolution, so will they permit unfortunately, super-biology in driving human cancers. This is the topic of this review.

Since this super-CG, super-hyperglycosylated CG driven process evolved to drive human evolution [35, 36]. It is important to understand human evolution first, before we consider human cancer, or a human evolution process gone haywire. The earliest primates, prosimian primates such as lemurs, had small brains, 0.07% of body weight (**Table 2**). This is because prosimian primates used inefficient non-invasive epitheliochorial placentation. With the evolution of the next level of primates, early simian primates such as platyrrhine or the new world monkey, CG and hyperglycosylated CG first evolved, and along with these molecules came primitive hemochorial placentation [35, 36]. Hemochorial placentation, or fetal circulation filtration by syncytiotrophoblast cell surrounded by maternal blood, is much more efficient.

In 1980 Fiddes and Goodman [37], examined the DNA sequence for the β-subunits of CG and LH in humans and primates, and showed that the evolution of CG from LH occurred by a single deletion mutation in LH β-subunit DNA and read -through into the 3'-untranslated region in early simian primates. In 2002 Maston and Ruvolo

Table 4. Use of urine ß-subunit core fragment as a tumor marker for detection of malignancies. Data from multiple reports (89-79,100-113).

Malignancy	Number of cases	Sensitivity (>3 fmol/ml)
Ovarian cancer	207	66%
Cervical cancer	410	48%
Endometrial cancer	157	47%
Pancreatic cancer	29	55%
Bladder cancer	102	48%
Lung cancer	122	24%
TOTAL	1027	Mean 48% detection

[38], investigated the DNA sequences of the β-subunit of CG in 14 primates and showed that the genes to make CG and its variants were not present in prosimians or primitive primates (example: Lemur), but evolved by the indicated deletion mutation with the early simian primates (platyrrhine or new world monkey). The first or early simian primates CG and hyperglycosylated CG molecules had just 3 N-linked and 2 O-liked oligosaccharides (**Table 2**). These evolved with the species about 37 million year ago (**Table 2**). With the evolution of advanced simian primates about 20 million ago (examples: orangutan and chimpanzee), with further point mutations a form of CG and hyperglycosylated CG evolved that had 3 N-linked and 3 O-linked oligosaccharides (**Table 2**). With the evolution of humans, approximately 0.1 millon year ago, and with further point mutations came the evolution of human CG and hyperglycosylated CG having 4 N -linked and 4 O-linked oligosaccharides. This increasing numbers of oligosaccharides and acidic sugars, 3 N-linked 2 O-linked, to 3 N-linked 3 O-linked and 4 N-linked 4 O-linked led to the evolution of a CG with an extreme acidity. Acidity ranged from pI 6.3 in early simians, to pI 4.8 in advanced simians and on to super-acidic pI 3.5 molecules in humans [35, 36, 38-40].

The metabolic clearance rate or circulating levels of CG were very much changed with acidity and evolution. As CG evolved with additional oligosaccharides containing sialic acid, it very much lengthened metabolic clearance rate of molecules and their effective bio-potency [38 41, 42]. As an example, at one extreme, regular human CG has 4 O-linked and 4 N-linked oligosaccharides all terminating in sialic acid residues. These acidify hCG resulting in a molecule with a mean isoelectric point (pI) of 3.5, and a circulating half-life of 36 hours or 2160 minutes [1]. At the other extreme, is LH (pI 9.0 [43]), the molecule that CG evolved from, has just 3 N-linked oligosaccharides. The metabolic clearance half-life of LH Is just 25 minutes [44], or 86 fold shorter that human CG. Human CG circulates for approximately 86 times longer than LH, raising the circulation concentration proportionately. A regression equation linking the number of oligosaccharides and the metabolic clearance rates them was formed. If clearance rate (minutes) half-life is CR and number of oligosaccharides is #O then $CR = (2.4^{\#O} \times 1.9)$. Using this equation it was calculated that that the clearance rate half-life of early simian primate CG was approximately 2.5 hours and the clearance rate half-life of advanced simian primate CG was approximately 6 hours.

The size of the brain in mammals is directly related to the combination of body mass and the metabolic support of the developing progeny [45]. The larger brain size, seen in advanced primates and humans, correlates with disproportionately large energy demands by the developing fetuses [45-51]. Numerous studies support the concept that advanced primates, and to a greater extent humans, had to develop more efficient or super efficient placentation mechanisms to support the increasing nutritional demands of their embryonic brain (**Table 2**) [39, 40, 45-55].

The prosimian primate had an average size mammalian brain, 0.07% of body mass (**Table 2**). In this species, epitheliochorial placentation was sufficient. Hemochorial placentation started with the evolution of CG in early simian primate. It was only with the appearance of CG and hyperglycosylated CG in early simian primates, that the signals to implant placentas inside the uterus [8, 9, 12-14], and the signals to generate villous placenta [36, 36], to promote angiogenesis of uterine vasculature [29-32] and development of the umbilical cord [33, 34] that hemochorial placentation happened [35]. Hemochorial placentation was primitive in early simian primates, implanting only through the depth of the decidua, leading to a larger brain 0.17% of body mass (**Table 2**). It was with evolution and the development of more-acidic more-potent CG and hyperglycosylated CG that hemochorial placentation went deeper to 1/10th myometrial depth in advance simian primates (**Table 2**). This supported the development of a much larger brain, 0.74% of body mass (**Table 2**).

With the evolution of humans and the multiple mutations needed to produce their super-CG with 2 additional oligosaccharides, hemochorial placentation went to the extreme. CG jumped in acidity from metabolic clearance rate half-life of 360 to 2160 minutes. With this hemochorial placentation went deeper to 1/3rd the thickness of the myometrium (**Table 2**). Hemochorial placentation reached the efficiency needed to support a human brain, 2.4% of body mass.

Nutrition transfer and placentation were taken to the extreme in the humans. Human CG has a circulating half-time of 2160 minutes. This leads to invasion to one third the thickness of the myometrium and to the super-efficient placentation that is needed to support the nutritional transfer necessary for a brain of 2.4% body mass or 3 fold greater than that of advanced simians [46, 48, 49, 55]. Considering the relationship between regular CG, hyperglycosylated CG and hemochorial placentation, and between advancing acidity of CG and advancing invasion and angiogenesis, it would not be unreasonable to claim that the evolution of CG in early simians started primates on the evolution path to advanced brains, or is at the root of human evolution [35, 36].

It is with these evolution stories that super-CG and super-hyperglycosylated CG or hCG and hyperglycosylated hCG were created, and this cancer story starts. Two potent growth promoters, normally reserved for evolution, and for pregnancy [35, 36]. Human cancers use hyperglycosylated hCG and its free subunit variants to drive the most efficient possible malignancy. It is at this point that this review starts.

HCG, one name shared by five independent molecules

Research in the last 10 years has shown that the molecule generally called human chorionic gonadotropin (hCG) is not one independent molecule, but rather is 5 separate molecules with independent functions. The five separate forms of hCG all share a common amino acid backbone, thus have a common name. They vary greatly, however, in carbohydrate side chain structure and meric structure (**Table 1**).

hCG is a hormone made by placental syncytiotrophoblast cells [7]. hCG comprises a 92 amino acid α-subunit and a 145 amino acid β-subunit. The β-subunit of hCG, while structurally similar to the β-subunit of LH, differentiates hCG from other glycoprotein hormones. hCG, like LH, is a hormone, and binds a common hCG/LH hormone receptor.

For the first 3 weeks of pregnancy, hCG promotes production of progesterone by ovarian corpus luteal cells [56-58]. Multiple research groups have shown that hCG also functions during pregnancy to promote angiogenesis in the uterine vasculature [29-32]. This insures maximal blood supply to the invading placenta, an important function during pregnancy. While hyperglycosylated hCG may promote cytotrophoblast cell growth during pregnancy [6, 9-14], hCG promotes the fusion of cells and their differentiation to syncytiotrophoblast cells [28]. It is the combination of these two processes that leads to villous trophoblast tissue formation and hemochorial placentation in pregnancy [35, 36]. Multiple groups show that hCG promotes an anti-macrophage inhibitory factor or a macrophage migration inhibitory factor that prevents destruction of the foreign feto-placental by the mother's tissue during pregnancy [59, 60]. Other groups have shown that hCG also controls uterine growth during pregnancy [61, 62], and yet other groups have shown that hCG also relaxes myometrial contractions during pregnancy [63, 64].

It has been shown that hCG also control umbilical cord growth and circulation and development during pregnancy [33, 34]. New research is finding receptors in fetal organs and a further role for hCG in fetal growth during pregnancy [65, 66].

The structure of the N-linked and O-linked oligosaccharide side chains attached to the hormone hCG are shown in **Figure 2**. The three dimensional structure of hCG dimer was shown by Lapthorn and colleagues (**Figure 1**) [2]. As shown, the β-subunit wraps itself around the α-subunit (**Figure 1**). Hyperglycosylation of hCG subunits leads to incomplete folding, this leads to exposure of sequences otherwise hidden on hCG. These are the evolutionary TGFβ structures. Hyperglycosylated hCG is an autocrine, and not a hormone like hCG, it seemingly binds and antagonizes TGFβ receptors on the cytotrophoblast cells that make hyperglycosylated hCG [6, 8-26]. This is part of the process of pregnancy implantation. Hyperglycosylated hCG promotes blockage of apoptosis in these cells, and production of collagenases and metalloproteinases needed for invasion in the implantation process [8-26]. Hyperglycosylated hCG also promotes cytotrophoblast cells or placental growth during the length of pregnancy [11-14].

Hyperglycosylated hCG drives invasion as occurs in the fastest growing human malignancy, choriocarcinoma. Classically, a women may have a normal pregnancy, and deliver with just a few cytotrophoblast cell remaining at the implantation site. Transformation may occur in one of these remaining cells. Just 6 to 10 weeks later, the new mother may show at an emergency room with difficulty breathing and seizures, due to choriocarcinoma spreading to her lungs, and in her brain. This is choriocarcinoma, a malignancy totally driven by hyperglycosylated hCG and seemingly by the TGFβ antagonism process normally reserved for pregnancy implantation [6, 9, 11, 27, 67, 68].

Choriocarcinoma is not the only malignancy that produces hyperglycosylated hCG, and uses hyperglycosylated hCG to drive its malignancy. Testicular and ovarian germ cell malignancies take on a cytotrophoblast histology and are driven by hyperglycosylated hCG [9, 27]. These are the only malignancies that misuse this evolution growth factor to drive their malignancy, hyperglycosylated hCG. As we now understand, all other malignancies use a similar TGFβ antagonism pathway when they can become advanced and can reach a state of differentiation whereby they express an hCGβ gene [69-79]. These cancer cells seemingly lack the ability to combine hCG subunits and just secrete hCGβ or hyperglycosylated hCGβ. Both of these molecules can antagonize the TGFβ receptor and promote malignancy [5, 6]. As now demonstrated, all advanced cancers are directly promoted to grow, invade and metastasize by an autocrine hCGβ or hyperglycosylated hCGβ [69-79]. Actions include inhibition of apoptosis in cancer cells and promotion of invasion proteases by cancer cells [41-48]. As demonstrated, recently, hyperglycosylated hCG, hCGβ and hyperglycosylated hCGβ are inter-changeable promoters, that all can promote choriocarcinoma or other advanced malignancies [6].

A fifth or final variant of hCG is made by pituitary gonadotrope cells during the menstrual cycle [80-83]. This is the sulfated variant of hCG with sulfated oligosaccharides as shown in **Table 1** and **Figure 2** [80]. Research by Odell and Griffin [81, 82] using an ultrasensitive hCG assay shows that sulfated hCG is produced during the length of the menstrual cycle, following the secretion pattern of LH. hCG and LH bind a common receptor. Research in Cole's laboratory shows that sulfated hCG production in 277 menstrual cycles at the time of the LH peak averages 1.54 ± 0.90 mIU/ml [83]. It appears that sulfated hCG matches LH function in promoting androstenedione production by theca cells, progesterone production by corpus luteal cells and in enhancing ovulation.

Choriocarcinoma and germ cell malignancies

Choriocarcinoma is a gestational trophoblastic disease, residing at the interface of obstetrics and oncology. Transformation in choriocarcinoma cases seemingly involves blockage of cytotrophoblast cells from fusing to form syncytiotrophoblast cells [9, 11, 27, 67, 68]. Cytotrophoblast cells are the site of hyperglycosylated hCG production, the driving force behind choriocarcinoma [9, 11].

The big question is what is the best tumor marker? Only one set of tumor markers fit this criterion, total hCG and hyperglycosylated hCG [67, 84-87]. Both of these tumor markers are 100% sensitive for choriocarcinoma. This is be-

cause choriocarcinoma cannot exist without hyperglycosylated hCG, as measured as hyperglycosylated hCG or total hCG immunoassays. No other tumor marker can make this claim. As demonstrated, when choriocarcinoma cells are grown in a nude mouse, they grow very rapidly. When an antibody is given to bind hyperglycosylated hCG, all growth completely stops [9, 11]. Similarly, when nude mice are administered choriocarcinoma cells in which the hCG subunit genes are blocked with anti-sense cDNA, all growth ceases [88, 89]. It is concluded that choriocarcinoma cannot exist without hyperglycosylated hCG.

The USA hCG Reference Service uses the B152 antibody hyperglycosylated hCG assay. This test detects hyperglycosylated hCG and its free β-subunit, hyperglycosylated hCGβ [90]. In the USA hCG Reference Service experience this tumor marker detects 100% of choriocarcinoma, persistent hydatidiform mole, testicular germ cell malignancy and ovarian germ cell malignancy cases. This test is diagnostic, it demonstrates malignant vs. quiescent or benign disease (<1% hyperglycosylated hCG) [85-87], in all these cancers. A company named Omnimmune Corp. (Houston. Texas) with its exclusive rights to B152 for cancer therapy, plans to humanize B152. This antibody possibly cures choriocarcinoma, persistent hydatidiform mole and testicular and ovarian germ cell malignancies

The first evidence that hyperglycosylated hCG functions in cancer through antagonizing a TGFβ receptor comes from the finding of evolutionary roots between hCGβ and TGFβ [3, 4], and from the finding of a 4 peptide cystine knot structure, common to hCG and TGFβ (and to nerve growth factor and platelet-derive growth factor) [2] (**Figure 1**). Multiple other articles show that the promotion of choriocarcinoma and pregnancy implantation (hyperglycosylated hCG), and metalloproteinase production, must be a TGFβ-mediated pathway [15-27]. Then there is the findings that the molecule which antagonized TGFβ in choriocarcinoma has the exact molecular size by polyacrylamide gel electrophoresis as hyperglycosylated hCG [26]. Finally, there is the demonstration that hyperglycosylated hCG, hCGβ and hyperglycosylated hCGβ are interchangeable, all competing with a TGFβ to bind a TGFβ receptor [5, 6]. It is inferred that hyperglycosylated hCGβ, hCGβ and hyperglycosylated hCG act though similar mechanisms, TGFβ receptor antagonism, to control apoptosis, to control cell growth, and promote collagenases and metalloproteinases promoting invasion [5, 6, 15-26].

The story with choriocarcinoma and germ cell malignancies does not stop here. Choriocarcinoma is an important part of cancer history. It has always been at the root of major discoveries. It was at the root of discovery of chemotherapy as a cure for cancer. As was known, choriocarcinoma is an extremely fast growing malignancy. As Dr. Roy Hertz reasoned, why doesn't an inhibitor of cell division or DNA synthesis block choriocarcinoma cancer growth. As reasoned, methotrexate blocks the synthesis of the critical DNA nucleotide thymidine. Why doesn't methotrexate block choriocarcinoma growth? As shown by Dr. Hertz in the nineteen fifties, methotrexate makes an effective treatment of choriocarcinoma [93-95]. This discovery led to modern chemotherapy treatment for cancer.

Now here, we start again with choriocarcinoma showing that hyperglycosylated hCG drives invasion seemingly through a TGF antagonism mechanism. We go on to show in the next chapter that this mechanism applies to most advanced malignancies. We state again that B152 hyperglycosylated hCG antibody treatment seeming stops choriocarcinoma dead in its tracks, as shown in nude mouse studies [11, 88, 89]. Research is suggesting that hCGβ and hyperglycosylated hCG antibody treatment could become the future of all cancer treatment.

Other malignancies

Here we consider all other malignancies, other than choriocarcinoma and germ cell malignancies. We consider all the common malignancies. Reports in the last 30 years show that most cancers, when advanced, produce an hCGβ immunoreactive substance [5, 69-79, 96-114].

As discovered in 1981 [96, 97], most other malignancies produce hCGβ or a large variant of hCGβ. In the following years, hundreds of research articles established that all human malignancies produced hCGβ or large variant of hCGβ [5, 69-79, 98-113]. hCGβ is detected in patient serum, or its degradation product β-core fragment is detected in urine. As shown recently by Valmu et al. [91], the large form of hCGβ is

actually hyperglycosylated hCGβ, a variant of hCGβ similar to the β-subunit of hyperglycosylated hCG. Why some cancers produce primarily hyperglycosylated hCGβ versus hCGβ is not known.

The literature shows that all advanced malignancies secrete hCGβ or hyperglycosylated hCGβ [98, 99], yet only a small proportion of malignancy cases, about 30%, have hCGβ or hyperglycosylated hCGβ in blood (**Table 3**), or their degradation product, β-core fragment present in urine of 48% of cancer cases (**Table 4**). This is because hCGβ and hyperglycosylated hCGβ are rapidly cleaved by the enzyme leukocyte elastase, produced my macrophages and leukocytes upon upon secretion. This enzyme first nicks or cleaves the molecules at β47-48 upon secretion, and then cleaves this molecule's C-terminal, or major acidic component by cleavage at β92-93 (**Figure 1**) [71, 115]. The resulting degradion products are rapidly cleared from the circulation by the liver and kidney, with circulating half lives of a few minutes verses 36 hour like hCG [71, 115]. This makes detection of the hCGβ or hyperglycosylated hCGβ in cancer cases very difficult, yielding a detection rate in blood of just 30%.

An accumulation of studies (**Tables 3** and **4**) shows that most malignancies produce this molecule [98, 99]. Urine β-core fragment is a useful tumor marker in gynecologic oncology, detecting 47% of endometrial, 48% of cervical and 66% of ovarian malignancies. Urine β-core fragment can be used as a simple three monthly screening test in women with familial ovarian cancer. Urine β-core fragment can be used as a wide spectrum cancer screening test. Yes, it detects 48% of all cancers, but a person positive in a β-core fragment assay can only then be screened with MRIs of the head and pelvis and chest CT to determine the site of malignancy.

Examination of the crystal structure of hCG [2], shows that the β-subunit has common evolutionary sequences with TGFβ [3, 4], and a cystine knot structure unique to hCG, TGFβ, platelet-derived growth factor and nerve growth factor. The site of this cystine knot structure is shown in **Figure 1**. As demonstrated [5], hCGβ antagonizes a TGFβ receptor site inhibiting apoptosis in the cancer cells, indicating that hCGβ, hyperglycosylated hCG and hyperglycosylated hCGβ antagonize this receptor [5, 6, 69, 70]. As reported, hCGβ and hyperglycosylated hCGβ promote the production of collagenases and metalloproteinases, invasion proteases produced by cancer cells [69], leading to metastases.

As shown recently [6], cancers other than choriocarcinoma and germ cell malignancies produce hCGβ and hyperglycosylated hCG. Hyperglycosylated hCG, hCGβ and hyperglycosylated hCGβ are all interchangeable. Just as hCGβ can do hyperglycosylated hCG's job with choriocarcinoma, so can hyperglycosylated hCG do hCGβ's job with other malignancies [6]. It appears that they all are interchangeable markers, all seemingly acting on a TGFβ receptor to antagonize it.

In recent years, hCGβ vaccines are being evaluated for patients with advanced cancers [116-121]. Initial clinical trials are extremely promising, showing a 2-fold extention of cancer survival [118-121]. The vaccine studies confirms the key role that hCGβ/hyperglycosylated hCGβ has in cancer metastasis and its action in all cancer cases.

It is my understanding that choriocarcinoma, persistent hydatidiform mole and germ cell malignancies are promoted by hyperglycosylated hCG in all stages. These are eutopic malignancies or malignancies driven by hyperglycosylated hCG. Hyperglycosylated hCG is seemingly the single cancer promoter, since cancer is brought to a complete halt in nude mice when hCG supply is blocked by antibody or DNA factors [11, 88, 89]. Other malignancies produce hCGβ and hyperglycosylated hCGβ. This is only produced in advanced disease [5, 69-79, 98-114]. It seems that the other or ectopic malignancies have to be advanced to differentiate tissues and to express ectopic hCGβ. From the time that hCGβ is ectopically expressed onwards hCGβ may be the principal driver of the malignancies. Based on the vaccine studies, it appears, as suggested [98, 99], that all malignancies may be controlled in advanced stages by hCGβ and/or hyperglycosylated hCGβ. It appears that once advanced malignancies start to express hCGβ and/or hyperglycosylated hCGβ that the malignancy may then be controlled by the TGFβ antagonism choriocarcinoma-like route by a molecule like hCGβ/hyperglycosylated hCGβ. It appears that hCGβ/hyperglycosylated hCGβ should be the target of much cancer research, it is the future, the mole-

cules that seemingly drive advanced malignancies.

The future

In conclusion, it appears that hyperglycosylated hCG, hCGβ and hyperglycosylated hCGβ are an inter-related set of molecules [6]. That seemingly drive cancer through a TGFβ antagonism pathway [5, 6, 23, 25]. Choriocarcinoma and germ cell malignances are all seemingly driven in early and advanced stages by this highly invasive pathway. In contrast, most other cancers are driven by alternative pathways until they become advance and express the hCGβ gene. They seeming adopt this viscous TGFβ antagonism pathway. This may be the key cancer physiology pathway.

This review presents research on cancers taking this pathway and promises for the future. Antibodies to hyperglycosylated hCG may seemingly cure choriocarcinoma and germ cell malignancies in the future, and vaccines to hCGβ and administered antibodies may significantly extend the lives of all advanced cancer patients. Vaccines may not work in some advanced stage cases, in patients with compromised immune systems. This is where administered antibodies may be most warranted.

In evolution, the molecule hyperglycosylated hCG was recruited to drive human evolution as an extreme growth factor. A growth factor that drove placental implantation deeper and growth to extremes. Unfortunately cancers take advantage of the availability of the extreme growth factors. It appears that the hCGβ/ hyperglycosylated hCGβ TGFβ pathway may be the central pathway to treatment of all advanced cancers.

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