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The Carbohydrate Structure of the Hormone hCG, the Autocrine Hyper Glycosylated hCG, and the Extravillous Cytotrophoblast Hyperglycosylated hCG

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Abstract

Objective: Carbohydrate structure has been determined for pregnancy hCG, hydatidiform mole hCG and choriocarcinoma hCG by multiple authors. It has not, however, been discriminated for the three hCG components, the hormone hCG, the autocrine hyperglycosylated hCG or for invasive cell hyperglycosylated hCG. This is determined here.

Methods: Three methodologies were used to discriminate the three components of hCG. The B152 immunoassay specifically determines the concentration of hyperglycosylated hCG. Matrigel invasion chambers determine invasiveness of first trimester trophoblast cell and extravillous cytotrophoblast cell preparations. Recombinant hCG is a marker of 100% hormone hCG.

Results: B152 immunoassay binds only hyperglycosylated hCG, and identified all hyperglycosylated hCG in pregnancy hCG, hydatidiform mole hCG and choriocarcinoma hCG preparations. Distribution of triantennary sugar structures same as percent invasion of Matrigel membrane, indicating distribution of invasive extravillous cytotrophoblast component. Recombinant hCG is only hormone hCG, contains 100% Type 1 O-linked oligosaccharides. Identifies Type 1 O-linked oligosaccharide hCG as hormone hCG.

Discussion: As determined, first trimester trophoblast cells produced 86.3% hormone hCG (biantennary N-linked and Type 1 O-linked oligosaccharides), 3.5% hyperglycosylated hCG (biantennary N-linked and Type 2 O-linked oligosaccharides) and 13.7% invasive extravillous cytotrophoblast hCG (triantennary N-linked and Type 2 O-linked oligosaccharides). Invasive extravillous cytotrophoblast cells produce 50.4% hormone hCG, 6.0% hyperglycosylated hCG and 42% invasive extravillous cytotrophoblast hCG. Hydatidiform mole cells produce 73.2% hormone hCG, 5.4% hyperglycosylated hCG and 23.4% invasive extravillous cytotrophoblast hCG. Choriocarcinoma cells produce 38.6% hormone hCG, 17.6% hyperglycosylated hCG and 42.8% invasive extravillous cytotrophoblast hCG.

Keywords: Hcg; Cytotrophoblast; Matrigel invasion assays; Immunoassays; Hyperglycosylated hCG

Introduction

Multiple authors have examined the carbohydrate structures of hCG [1-4]. All examined purified pregnancy hCG, hydatidiform mole hCG and choriocarcinoma hCG, and not the proven components of hCG, the hormone hCG, the autocrine hyperglycosylated hCG, and the invasive cell hyperglycosylated hCG. Unfortunately, all 3 separate hCG forms, while having separate functions [5-8], are all approximately the same size, molecular weight 36500, 37400, 38800 respectively and the same charge making them un-separable.

Here, for the first time, hCG carbohydrate structures were examined [3] and explore other evidence, to show the different carbohydrates present on the hormone hCG, the autocrine hyperglycosylated hCG and the invasive cell hyperglycosylated hCG.

Materials and Methods

Culture procedures

Purified term primary cytotrophoblast cells from term pregnancies were kindly provided by Harvey Kliman at Yale University in Dulbecco's High Glucose medium with 10% fetal calf serum (DHG-10%). Cytotrophoblast cell were purified by Percoll density centrifugation from trypsin dispersed term pregnancy villous trophoblast tissue using the methods used by Harvey Kliman previously [9]. Spent culture fluid was tested for total hCG and hyperglycosylated hCG.

Culture medium was collected from 70% confluent flasks of line and ACH-3P normal first trimester placenta cell line. Cells were cultured to confluency in (Roswell Park Memorial Institute medium) RPMI-1640 medium with 10% fetal calf serum (RPMI-10%). Spent culture fluid was tested for total hCG and hyperglycosylated hCG.

Pure Sample		α-subunit N-linked oligosaccharides (%)							ß-subunit N-linked oligosaccharides (%)							O-linked oligosaccharides (%)		
		GM	GGF	GGM	GG	GGGF	GGG	Sialic Acid (pmol/pmol)	GM	GGF	GGM	GG	GGGF	GGG	Sialic acid (pmol/pmol)	Type 1	Type 2	Sialic acid (pmol/pmol)
Pregnancy	CR127 pool	48	8.9	0	38.4	0	4.8	1.5	3.6	56.3	0	34.7	5.4	0	1.91	81.5	18.5	1.32
	CR129 pool	49.7	3.1	0	37.8	0	9.4	1.47	2.2	59.3	0	27.7	10.8	0	2.01	84.4	15.6	1.25
	Individual P3	53.8	7.3	0	36.4	2.6	0	1.76	6.5	44.3	0	38.9	8.3	2.1	2	87.7	12.3	1.62
	Individual P7	40.7	7.9	0	37.1	14.3	0	1.7	4.4	54.9	0	23	13.2	4.5	2.32	81	19	1.02
	Individual P8	41.5	7.3	0	41.1	10.1	0	1.73	1.9	51.9	0	24.6	17.4	4.2	2.06	87.5	12.5	1.46
	Individual P9	61.9	9.4	0	29.2	0	0	na	8.8	38.2	0	36.4	12.1	4.4	2.03	84.6	15.4	0.88
Serono recombinant hCG		52.2	4.8	0	43	0	0	na	5.3	57.5	0	37.2	0	0	na	100	0	na
Extravillous cytotrophoblast		39.5	15	4.5	18	6.9	16	1.8	15	24.4	0	11	35.6	6.4	na	52	48	na
Hydatidiform Mole	Individual M1	43.4	16.7	0	32.3	3.6	3.9	1.32	5.5	57.2	0	19.1	16.5	1.7	2.1	88.9	11.1	0.45
	Individual M2	53.1	4.4	0	36.6	0	5.9	1.38	6.9	38.4	0	22.6	20.9	11.1	2.1	61.9	38.1	0.8
	Individual M4	35.7	0	0	54.6	0.5	9.2	1.39	8.1	22.8	0	39	18.5	11.5	2.66	80.3	19.7	0.98
Choriocarcinoma	Individual C1	38.5	16	3.6	18.9	6.2	16.7	1.7	6.8	14.8	0	14.8	52.2	11.4	2.37	33.4	66.6	0.52
	Individual C2	56.6	24.2	3.7	5.7	0	9.8	1.36	5.9	23.4	0	22.6	38	10.1	1.82	52.1	47.9	1.14
	Individual C3	71.1	19.1	2.2	4.9	0	2.9	1.06	16.4	23.8	0	11.7	33.6	14.5	2.36	11.8	88.2	0.97
	Individual C5	74.8	18.2	2.3	3.5	0	1.2	0.92	15.9	31.9	0	4.4	42.7	5.1	2.19	0	103	1.15
	Individual C7	56.5	20.3	5.1	6.8	8	4.3	0.98	10.9	24.4	9.1	7.5	35.2	12.9	1.6	31.9	68.1	1.05

Table 1: The oligosaccharide content of hCG [3] (na is not determined. GM is oligosaccharides terminating in Galactose (Gal) and Manose (Man); GGF in Gal, Gal and Fucose (Fuc); GGM in Gal, Gal and Man; GG in Gal and Gal; GGGF in Gal, Gal, Gal and Fuc; GGG in Gal, Gal and Gal. CR127 and CR129 are batches of hCG standards).

Matrigel invasion assays

Primary third trimester cytotrophoblast cells, and ACH-3P first trimester trophoblast cells were harvested with trypsin and EDTA. Cells were plated in quadruplicate onto Matrigel membranes and control inserts, 5000 cells per membrane and per control insert (Biocoat Matrigel invasion membranes, BD Biosciences, Bedford, MA 01730). Cells were cultured at 37°C for 24 h in DHG-10% culture fluid containing no additives (controls), and with 10 and 100 ng/mL C5 hyperglycosylated hCG on quadruplicate membranes. Matrigel membranes were processed and percentage invasion calculated as suggested by the manufacturer in package inserts. Briefly, membranes are rehydrated in DHG-10% in the incubator for 2 h before use. Membranes and control inserts are then plated (25,000 cells in 0.5 medium per plate). Plates are cultured for 24 h, and membranes removed from the inserts using a scalpel. Membranes are transferred to a slide using Cytoseal mounting medium (Stephens Scientific Inc., Riverdale NJ), exposing the under surface or the invaded cells. Cells are stained with DIF-Quick Stain (IMEB Inc., Chicago IL) to mark nuclei. Invaded cells are counted at 5 marked places, and the count averaged. Cell penetration or invasion of membranes is directly compared to that of correspondingly cultured control inserts and the

percentage invasion is calculated using the formula provided by the manufacturer.

Immunoassays

Total hCG was measured with the Siemens Immulite 1000 automated hCG immunoassay. A Serono (Geneva, Switzerland) recombinant hCG standard curve was run, 0.1, 0.3, 1.0, 3.0 and 10 ng/mL. The assay was run exactly as described by Siemens Healthineers (Erlangen, Germany), the manufacturer. The Immulite 1000 assay detects hormone hCG, hyperglycosylated hCG, nicked hCG and dissociated hCG β-subunit equally.

Hyperglycosylated hCG was measured in the microtiter plate assay using antibody B152 as described previously [3]. This antibody only detects hyperglycosylated hCG and hyperglycosylated hCG β-subunit [10].

Results and Discussion

Table 1 shows the carbohydrate structure in pregnancy hCG, hydatidiform mole hCG and choriocarcinoma hCG as shown by Elliott MM, Kardana A, Lustbader JW and Cole LA [3], the most comprehensive hCG carbohydrate structure report (covering the largest number of samples). This is the data which was extracted, the proportion of carbohydrate structures on the three forms of hCG. Monoclonal antibody B152 was raised against C5 hyperglycosylated hCG, a preparation of 100% hyperglycosylated hCG. The B152 test site includes β-subunit O-linked oligosaccharide at β121. The antibody binds hyperglycosylated hCG and its β-subunit but not the hormone hCG or its β-subunit [10]. Antibody B152 was used to measure the amount of hyperglycosylated hCG in each hCG sample (Table 2). As shown, the amount of hyperglycosylated hCG almost exactly matches the proportion of molecules with Type 2 O-linked structures.

The proportion of B152 active hCG molecules is assumed to be the total proportion hyperglycosylated hCG. It was noticed that the remainder included a proportion of triantennary N-linked structures, these were 13.7 ± 5.9% of first trimester pregnancy hCG and 42% of fresh term pregnancy cytotrophoblast cell hCG. Multiple authors have reported that there are two distinct kinds of cytotrophoblast cells, normal cytotrophoblast cell and invasive extravillous cytotrophoblast cells [11-17]. Extravillous cyotrophoblast cells are the invasive cell of blastocyst implantation and deep implantation of pregnancy [18,19], and the cells that link hemochorial placentation to the uterus [20]. Invasive extravillous cytotrophoblast can be measured as sorted term primary cytotrophoblast cells [9]. Matrigel invasion study was performed to examine the source of the triantennary N-linked oligosaccharides. First trimester ACH-3P cells, 12 ± 2.4% invaded the mouse sarcoma basement membrane and 40 ± 10% of extravillous cytotrophoblast cells (term pregnancy primary cytotrophoblast cells) invaded the membrane (Table 3).

Sample	B152 assay as a percentage of total hCG (Siemens Immulite assay)	Percentage Type 2 (O-linked oligosaccharides from Table 1)
CR127 pooled pregnancy hCG	0.075	0.054
CR129 pooled pregnancy hCG	0.12	0.108
Individual P3 Pregnancy hCG	0.094	0.123
Individual P7 Pregnancy hCG	0.175	0.19
Individual P8 Pregnancy hCG	0.127	0.125
Individual P9 Pregnancy hCG	0.175	0.154
Individual M1 Hydatidiform mole hCG	0.114	0.111
Individual M2 Hydatidiform mole hCG	0.373	0.381
Individual M4 Hydatidiform mole hCG	0.25	0.197
Individual C1 Choriocarcinoma ChoriocarcinomaChoriocarcinoma hCG	0.7	0.666
Individual C2 Choriocarcinoma hCG	0.32	0.479
Individual C3 Choriocarcinoma hCG	0.87	0.882
Individual C5 Choriocarcinoma hCG	1.08	1.03
Individual C7 Choriocarcinoma hCG	0.64	0.681

Table 2: The proportion of hCG detected by the B152 hyperglycosylated hCG assay.

		Penetration of Matrigel Membranes mean ± SD OR % triantennary oligosaccharides mean ± SD
Study 1: Cole Matrigel study, % invasion	ACH-3P First trimester trophoblast cell	12 ± 2.4%
	Extravillous cytotrophoblast (fresh term cytotrophoblast primary cells)	40 ± 10 %
Study 2: Cole carbohydrate study, % triantennary structures	First trimester pregnancy [19]	13.7 ± 5.9%
	Extravillous cytotrophoblast (fresh term cytotrophoblast primary cells) studies	0.42

Table 3: Proportion of invasive cells. Three studies were performed to determine the propotion of invasive cells (%). Study 1 used Matrigel membranes, proportion of cells penetrating a mouse sarcoma basement membrane (mean% ± standard deviation) to estimate proportion of invasive cells [12]. Study 2 is carbohydrate structure, it uses the published distribution of triantennary oligosaccharide to indicate the proportion of invasive cells [20].

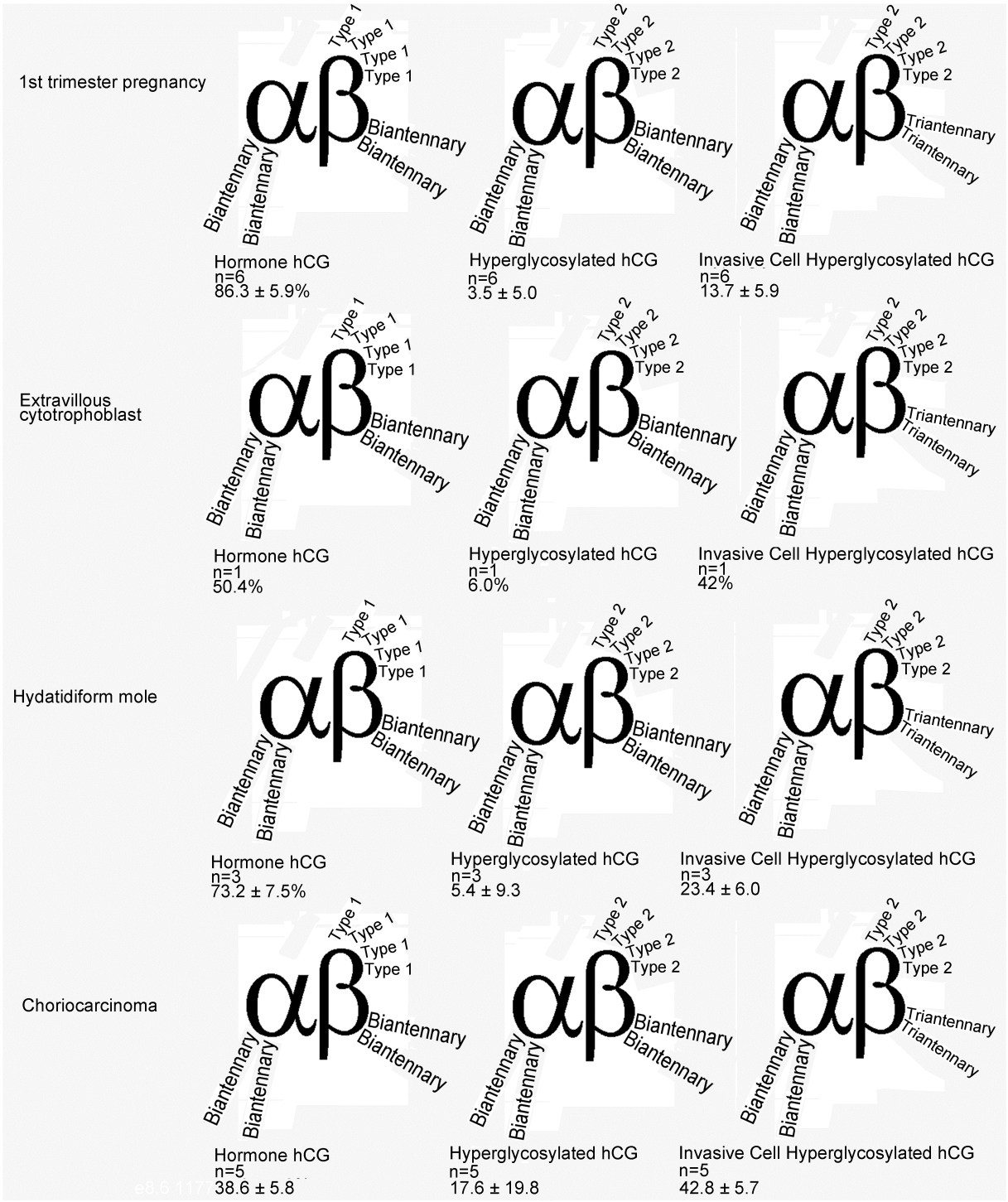

Figure 1: Distribution of hCG sugars.

Clearly the proportion of triantennary structures in first trimester pregnancy or and extravillous cytotrophoblast cells, 13.9 ± 5.9% and 42%, matches almost exactly the Matrigel data, 12 ± 2.4% and 40 ± 10% for the proportion of Matrigel invasive cells, showing that the triantennary structures are for extravillous cytotrophoblast cell hCG. It was assumed accordingly that the triantennary N-linked oligosaccharide hCG represented the extravillous cytotrophoblast hCG.

The remainder of the first trimester pregnancy, extravillous cytotrophoblast, hydatidiform, and choriocarcinoma hCG carbohydrate structure was assumed to be the hormone hCG. This is

based on the remaining composition, Type 1 O-linked oligosaccharides and biantennary B-linked oligosaccharides being that of Serono recombinant hCG which is 100% the hormone hCG.

Are any of these test useful in medical practice. The B152 immunoassay, now is available at Quest Diagnostics may be useful in determining the proportion hyperglycosylated hCG in the hCG mixture. This may be useful in examining choriocarcinoma serum samples and hydatidiform mole serum samples to determine the ratio of cells present, cytotrophoblast (produce autocrine hyperglycosylated hCG) and syncytiotrophoblast (produce hormone hCG) cells. As for using Matrigel clinically, this is strictly a test for cell invasiveness, and may or may not have an application in the laboratory.

Conclusion

Putting all the data together, in conclusion, all preparations, whether first trimester trophoblast, extravillous cytotrophoblast, hydatidiform mole or choriocarcinoma are a mixture of hormone hCG (biantennary N-linked with Type 1 O-linked oligosaccharides), hyperglycosylated hCG (biantennary N-linked with type 2 O-linked oligosaccharides) and extravillous cytotrophoblast hyperglycosylated hCG (triantennary N-linked on β-subunit with Type 2 O-linked oligosaccharides) (Figure 1), three distinct structures.

As shown in Figure 1, first trimester trophoblast cells produced 86.3% hormone hCG (biantennary N-linked and Type 1 O-linked oligosaccharides on β-subunit), 3.5% hyperglycosylated hCG (biantennary N-linked and Type 2 O-linked oligosaccharides on β-subunit) and 13.7% invasive extravillous cytotrophoblast hCG (triantennary N-linked and Type 2 O-linked oligosaccharides on β-subunit). Invasive extravillous cytotrophoblast cells produce 50.4% hormone hCG, 6.0% hyperglycosylated hCG and 42% invasive extravillous cytotrophoblast hCG. Hydatidiform mole cells produce 73.2% hormone hCG, 5.4% hyperglycosylated hCG and 23.4% invasive extravillous cytotrophoblast hCG. Choriocarcinoma cells produce 38.6% hormone hCG, 17.6% hyperglycosylated hCG and 42.8% invasive extravillous cytotrophoblast hCG.

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