hCG attenuates hyperglycosylated hCG-driven growth and invasion.

Laurence A. Cole*

USA hCG Reference Service, Angel Fire, NM 87710, USA

Abstract

Objective: To determine the role of the hormone hCG in pregnancy, choriocarcinoma and cancer cell growth and invasion.

Methods: Third trimester placenta cytotrophoblast primary cultures, two choriocarcinoma cell lines, and one trophoblastic cancer and 5 non-trophoblastic cancer cell lines.

Results: Hyperglycosylated hCG promotes growth and invasion of normal pregnancy, 2 of 2 choriocarcinoma cell lines and 6 of 6 cancer cell lines. The hormone hCG attenuates or blocks growth and invasion in normal pregnancy, 2 of 2 choriocarcinoma and 6 of 6 cancer cell lines.

Discussion: Cancer cells seemingly have adopted the hCG/LH receptor system. All cancers may be treated with hormone hCG to block and attenuate cancer growth, invasion and progression.

Keywords: Pregnancy failures, Matrigel membranes, Hyperglycosylated.

Accepted on January 10, 2018

Introduction

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by placental cells. hCG is in fact two separate independent molecules that are 97% structurally the same [1,2]. They have identical a- and β-subunit amino acid sequence [1,2], they share identical N-linked oligosaccharides, and very different type 1 and type 2 O-linked oligosaccharides on the C-terminal peptide of the β-subunit [2]. There is the hormone hCG produced by fused syncytiotrophoblast cells that binds and acts on a luteinizing hormone (LH)/hCG joint receptor on cytotrophoblast cells and on maternal uterine tissues and maternal corpus luteal cells [3,4]. And there is the autocrine hyperglycosylated hCG which is made by cytotrophoblast cells which has no action on the LH/hCG hormone receptor and antagonizes a transforming growth factor-β (TGF-β) receptor on cytotrophoblast cells [5-7].

It has been well established that hyperglycosylated hCG drives placental invasion as in implantation of pregnancy [8-10], and that it also drives choriocarcinoma cell growth and invasion and cancer cell grow and invasion or malignancy [8-12]. It achieves this through promoting the transferring growth factor-β (TGF-β) receptor and promoting production of collagenases and metalloproteinases [5-7,13-15].

Materials and Methods

Culture procedures

Purified 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 [16].

JAR and JEG-3 choriocarcinoma cell lines, NTERA testicular germ cell cancer cell line, ScaBER bladder carcinoma cells line, T24 epithelial bladder cancer cell line, CaSki epidermoid cervical cancer cell line, Hec-1-a endometrial adenocarcinoma cell line, and KLE endometrial aneuploid cancer cell line were all cultured to 70% flask confluency in T75 flasks with DHG-10% medium.

All flasks were cultured with supplemental hormone hCG (recombinant, Sigma, St. Louis MO) and supplemental hyperglycosylated hCG (batch C5) at 10 ng/ml, 100 ng/ml and 1,000 ng/ml using the same medium.

Immunoassays

Total hCG (all forms of hCG, including hCG-H, hCG and their free β-subunits) were measured using the Siemens Healthineers Inc. (New York, NY) Immulite hCG assay on the Immulite automated immunoassay platform. This assay was calibrated with recombinant hormone hCG in ng/ml. The sensitivity of this assay was 0.091 ng/ml, the equivalent of 1.0 mIU/ml assuming that 1 ng/ml=11 mIU/ml [5].

Hyperglycosylated hCG was measured using the established B152 microtiter plate immunometric assay [5], nicked hCG using the B151 microtiter plate immunometric assay, free β-subunit using the FBT11 microtiter plate immunometric assay [5] and β-core fragment using the B210 microtiter plate immunometric assay [5].

Matrigel invasion assay

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.

USA hCG reference service customers

All customers of the USA hCG Reference Service completed a form giving the USA hCG Reference Service permission to blindly publish total hCG and hyperglycosylated hCG results, and to use them for research. Three clients refused this option, their results are omitted from this report.

Pregnancy failures

Serial first morning urine samples were collected from 149 volunteer women all married, of mixed race (111 White, 28 Hispanic, 10 African American) and mixed age (range 18-36, mean 27.8 years) and all agreed to be actively involved in intercourse, particularly around the mid-cycle of the menstrual period, or all were eager to achieve to reach pregnancy. All female volunteers lived in Albuquerque NM. Urine collection was carefully monitored by University of New Mexico Human Research Review Committee (HRRC) the institutional review board (IRB) in approved application 04-132 signed 7/02/04.

In each case first morning urines were collected daily, and stored in supplied storage cups and immediately frozen in their home freezer. Urines were picked up at homes twice weekly and daily LH, total hCG and hyperglycosylated was measured. Women received $100 monthly for compensation for collecting daily first morning urines. All urine collection was sponsored by Church and Dwight Inc. and eventual pregnancy urines used were collected and sent to Church and Dwight to evaluate home pregnancy testing devices. Daily pregnancy urines were tested for total hCG and hyperglycosylated hCG from 2 days prior to implantation to 34 days of gestation. Church and Dwight Inc. approved this report as a side project.

The day of implantation of pregnancy was assumed as the day of production of the hormone hCG (sensitivity 0.091 ng/ml=1 mIU/ml) as proposed by Wilcox et al. [17].

Results

When normal placenta cytotrophoblast primary cells were cultured in Matrigel invasion chambers, cells invaded the plasma membrane chambers driven by the hyperglycosylated hCG produced by the cell line (Table 1, control cultures). They invaded by 41 ± 12%. When hyperglycosylated hCG was added to normal placenta cytotrophoblast cells, 10 ng/ml, invasion was extended to 66 ± 13%, and when 100 ng/ml was added invasion was extended to 86 ± 12%. The hormone hCG very much undid or attenuated this invasion, the hormone hCG, 10 ng/ml, diminished invasion to 34 ± 9%, and 100 ng/ml diminished invasion to 26 ± 11% (Table 1).

I next investigated hyperglycosylated hCG and hormone hCG action on primary cytotrophoblast cell growth (Table 2). Control cultures grew over 24 h to 543,000 ± 78,000 cells. When media was supplemented with 10 ng/ml hyperglycosylated hCG cells grew to 783,000 ± 67,000 (+41%) cells, when supplemented with 100 ng/ml they grew to 875,000 ± 72,000 cells (+61%). The hormone hCG very much undid or attenuated cell growth, 10 ng/ml, diminished growth to 424,000 ± 7,700 (-22%), 100 ng/ml diminished growth to 260,000 ± 11,000 (-52%) and 1,000 ng/ml diminished growth to 136,000 ± 10,000 (-75%) (Table 2).

Table 1. *Effect of hyperglycosylated hCG (hCG-H) and the hormone hCG on cell invasion on Matrigel membranes. Cells were separately cultured for 24 hours (5000 cells) on Matrigel basement membranes and control inserts in triplicate. Concentration of the hyperglycosylated hCG and the hormone hCG added to enhance or diminish invasion were 10 ng/ml and 100 ng/ml. The underside of Matrigel basement membranes, containing penetrated or invaded cells, was stained and counted. Cell penetration was compared with that of control inserts. Experiment performed in quadruplicate. The percentage penetration or invasion was calculated using the formula described by the manufacturer.*

	Penetration of Matrigel Membranes, mean ± SD
Pregnancy term placenta primary cytotrophoblast cells	
Control cultures, no additive	41 ± 12%
hCG-H, 10 ng/ml	66 ± 13%[a]
hCG-H, 100 ng/ml	86 ± 12%[a]
Hormone hCG, 10 ng/ml	34 ± 9%
Hormone hCG, 100 ng/ml	26 ± 11%[a]
JEG-3 Choriocarcinoma cytotrophoblast cells	
Control cultures, no additive	48 ± 11%
hCG-H, 10 ng/ml	68 ± 14%[a]
hCG-H, 100 ng/ml	88 ± 6%[a]
Hormone hCG, 10 ng/ml	42 ± 9%
Hormone hCG, 100 ng/ml	38 ± 3%[a]
JAr Choriocarcinoma cytotrophoblast cells	
Control culture, no additive	42 ±13%
hCG-H, 10 ng/ml	70 ± 11%[a]
hCG-H, 100 ng/ml	86 ±10%[a]
Hormone hCG, 10 ng/ml	32 ± 10%
Hormone hCG, 100 ng/ml	26 ± 8%[a]

[a]A significant difference was observed by t test compared to control, P >0.05

Table 2. *Promotion of cell growth by hyperglycosylated hCG, blockage or attenuation of cell growth by the hormone hCG. The abbreviation hCG-H is hyperglycosylated hCG, and hCG is the hormone hCG. Experiment performed in quadruplicate.*

	Control 0 ng/ml	hCG-H 10 ng/ml	hCG-H 100 ng/ml	hCG 10 ng/ml	hCG 100 ng/ml	hCG 1000 ng/ml
Pregnancy term placenta primary cytotrophoblast cells	543,000 ± 78,000	763,000 ± 67,000	873,000 ± 72,000	424,000 ± 7,700	260,000 ± 11,000	136,000 ± 10,000
	100%	+41%[a]	+61%[a]	-22%[a]	-52%[a]	-75%[a]
Jar choriocarcinoma	428,000 ± 45,000	478,000 ± 59,000	556,000 ± 33,000	343,000 ± 31,000	312,000 ± 57,000	127,000 ± 6,000
	100%	+12%[a]	+30%[a]	-20%[a]	-27%[a]	-70%[a]
JEG-3 choriocarcinoma	829,000 ± 45,000	912,000 ± 87,000	1,061,000 ± 28,000	698,000 ± 119,000	504,000 ± 71,000	361,000 ± 32,000
	100%	+10%[a]	+28%[a]	-16%[a]	-39%[a]	-56%[a]
NTERA testicular germ cell malignancy	446,000 ± 83,000	526,000 ± 18,000	589,000 ± 27,000	147,000 ± 14,000	134,000 ± 4,000	131,000 ± 4,000
	100%	+18%[a]	+32%[a]	-0.67	-70%[a]	-70%[a]
ScaBER bladder carcinoma	492,000 ± 10,000	738,000 ± 45,000	772,000 ± 19,000	400,000 ± 40,000	235,000 ± 17,000	199,000 ± 11,000
	100%	+50%[a]	+56%[a]	-19%[a]	-52%[a]	-60%[a]
T24 Epithelial bladder cancer	1,359,000 ± 60,000	1,494,900 ± 78,000	1,739,000 ± 227,000	1,150,000 ± 61,000	1,054,000 ± 35,000	473,000 ± 36,000
	100%	+10%[a]	+29%[a]	-15%[a]	-22%[a]	-66%[a]
CaSki epidermoid cervical cancer	839,000 ± 43,000	988,000 ± 82,000	1,256,000 + 125,000	736,000 ± 55,000	665,000 ± 22,000	479,000 ± 31,000
	100%	0.18	+50%[a]	-0.12	-21%[a]	-43%[a]
KLE endometrial aneuploid cancer	602,000 ± 57,000	704,000 ± 34,000	795,000 ± 7,000	451,000 ± 36,000	444,000 ± 18,000	400,000 ± 22,000
	100%	+17%[a]	+32%[a]	-25%[a]	-26%[a]	-34%[a]
Hec-1-a endometrial adenocarcinoma	578,000 ± 63,000	798,000 ± 58,000	959,000 ± 44,000	525,000 ± 58,000	350,000 ± 40,000	259,000 ± 18,000
	100%	+38%[a]	+66%[a]	-9%[a]	-39%[a]	-55%[a]

[a]A significant difference was observed by t test compared to control, P >0.05

It was concluded that hyperglycosylated hCG expanded primary placental cytotrophoblast cell growth and invasion, and that the hormone hCG attenuated placenta cytotrophoblast cell growth and invasion.

A total of 149 women volunteers tried to get pregnant over a 5 menstrual period, time. Of these 117 women achieved pregnancy, 73 achieved term pregnancy and childbirth, nineteen achieved a spontaneous abortion or miscarriage in the first trimester of pregnancy, 21 achieved a biochemical pregnancy or a miscarriage two or three days after implantation and 4 achieved an ectopic pregnancy or failing pregnancy outside of the uterus (Table 3).

The proportion hCG (hyperglycosylated hCG/ hyperglycosylated hCG+hormone hCG) forms among those with term/childbirth pregnancy was 69 of 73 (95%) >40%, mean is 112% (Table 3). The proportion hCG forms among those with spontaneous abortion of miscarriage pregnancies was 0 of 19 (0%) pregnancies >40%, mean is 14% indicating that no pregnancy made enough hyperglycosylated hCG for implantation, or all too much hCG for implantation. The proportion hCG forms among the 21 with biochemical pregnancies, 0 of 21 (0%) pregnancies made >40%, mean is 2.6% indicating once again that none made enough hyperglycosylated hCG or made too much hCG for implantation. Again with ectopic pregnancy 0 of 4 (0%) made >40% hyperglycosylated hCG needed for implantation (Table 3). The proportion hyperglycosylated hCG in these 117 pregnancies, 0 of 44 (0%) failing pregnancies produced >40% hyperglycosylated hCG, clearly shows the importance of sufficient hyperglycosylated hCG and the importance of not too much attenuating hCG for implantation to be complete.

Does hyperglycosylated hCG drive invasion and the hormone hCG attenuate invasion in choriocarcinoma? As shown in Table 1, using two choriocarcinoma cell lines, Jar and JEG-3, hyperglycosylated hCG drives invasion. In JEG-3 cells 10 ng/ml and 100 ng/ml hyperglycosylated hCG drove cells from 48 ± 11% invasion to 68 ± 14% to 88 ± 6%, while 10 ng/ml and 100 ng/ml hormone hCG diminished cells from 48 ± 11% to 42 ± 9% to 38 ± 3%. In Jar cells 10ng/ml and 100 ng/ml hyperglycosylated hCG drove cells from 42 ± 13% invasion to

70 ± 11% to 86 ± 10%, while 10 ng/ml and 100 ng/ml hormone hCG diminished cells from 42 ± 13% to 32 ± 10% to 26 ± 8%.

Table 3. *Proportion hyperglycosylated hCG in 117 pregnancies on the day of implantation, 73 term/childbirth pregnancies, 19 first trimester spontaneous abortion/miscarriage, 21 biochemical and 4 ectopic pregnancies. The abbreviation T-hCG is total hCG and H-hCG is hyperglycosylated hCG.*

Term/Childbirth pregnancy, n=73			**Spontaneous abortion pregnancy (n=19)**			**Biochemical pregnancy (N=21)**			**Ectopic pregnancy (n=4)**		
T-hCG	H-hCG	%	T-hCG	H-hCG	%	T-hCG	H-hCG	%	T-hCG	H-hCG	%
1.77	0.29	0.16	0.39	0.02	0.05	0.16	0	0	0.17	0.02	0.12
0.22	0.07	0.31	0.4	0.03	0.08	0.16	0	0	0.15	0.02	0.14
0.3	0.1	0.33	0.22	0.02	0.09	1.08	0.02	0.002	0.09	0.02	0.22
0.5	0.17	0.34	0.11	0.01	0.09	0.48	0.03	0.006	0.18	0.04	0.22
0.4	0.16	0.4	0.21	0.02	0.1	0.38	0.03	0.007			
0.22	0.09	0.4	0.1	0.01	0.1	0.19	0.02	0.009			
0.2	0.08	0.4	0.2	0.02	0.1	0.46	0.06	0.012			
0.22	0.09	0.4	0.09	0.01	0.11	0.19	0.02	0.015			
0.42	0.17	0.41	0.09	0.01	0.11	0.21	0.06	0.026			
0.21	0.09	0.43	0.16	0.02	0.12	0.15	0.05	0.026			
1.03	0.46	0.45	0.5	0.07	0.14	0.15	0.04	0.027			
0.11	0.05	0.46	0.13	0.02	0.16	0.11	0.03	0.028			
0.22	0.1	0.46	0.65	0.11	0.17	0.09	0.03	0.028			
0.5	0.23	0.46	0.12	0.02	0.17	0.11	0.03	0.028			
1.31	0.68	0.52	0.12	0.02	0.17	0.19	0.07	0.033			
0.42	0.22	0.52	0.11	0.02	0.18	0.1	0.04	0.034			
0.19	0.1	0.52	0.09	0.02	0.22	0.3	0.11	0.035			
1.02	0.54	0.53	0.09	0.02	0.22	0.25	0.09	0.035			
0.18	0.1	0.55	0.15	0.04	0.28	0.27	0.11	0.035			
0.45	0.27	0.59				0.11	0.05	0.038			
0.34	0.2	0.61				0.09	0.12	0.117			
0.25	0.15	0.61									
0.55	0.35	0.63									
0.15	0.1	0.65									
0.2	0.13	0.65									
0.18	0.12	0.66									
0.15	0.1	0.69									
0.15	0.1	0.69									
0.15	0.1	0.69									
0.11	0.08	0.7									
0.16	0.12	0.71									
0.15	0.11	0.71									
0.11	0.08	0.73									

0.14	0.1	0.73
0.14	0.1	0.73
0.39	0.29	0.74
0.81	0.61	0.75
0.13	0.1	0.79
0.32	0.26	0.82
0.12	0.1	0.85
0.12	0.1	0.85
1.48	1.32	0.89
0.11	0.1	0.92
0.11	0.1	0.92
0.23	0.21	0.94
0.23	0.21	0.94
0.48	0.46	0.96
0.3	0.29	0.97
0.21	0.21	1
0.11	0.11	1.02
0.62	0.65	1.05
0.49	0.52	1.06
0.53	0.58	1.09
0.29	0.32	1.1
0.09	0.1	1.1
0.28	0.32	1.12
0.13	0.15	1.2
0.08	0.1	1.22
0.37	0.49	1.31
0.35	0.51	1.46
1.08	1.74	1.62
0.12	0.22	1.86
0.27	0.52	1.9
0.1	0.2	1.95
0.19	0.45	2.37
1.03	2.5	2.43
0.24	0.62	2.62
0.14	0.37	2.71
0.27	0.77	2.82
0.15	0.43	2.96
1.3	4.7	3.61
0.09	0.43	4.74

0.09	0.56	6.13									
0.36	**0.32**	**112%**	**0.21**	**0.03**	**14%**	**0.25**	**0.05**	**2.60%**	**0.15**	**0.03**	**17%**

In terms of choriocarcinoma cell growth, in JEG-3 cells, hyperglycosylated hCG, 10 ng/ml and 100 ng/ml, developed cells by +10% and +28% (Table 2). Hormone hCG, 10 ng/ml, 100 ng/ml and 1,000 ng/ml diminished cell growth -16%, -39%, and -56%. Looking at Jar choriocarcinoma cells, hyperglycosylated hCG, 10 ng/ml and 100 ng/ml, developed cells by +12% and +30% (Table 2). Hormone hCG, 10 ng/ml, 100 ng/ml and 1,000 ng/ml diminished cell growth -20%, -27% and -70%.

Clearly, hyperglycosylated hCG promotes cell invasion and cell growth while the hormone hCG attenuates or blocks cell invasion and cell growth in choriocarcinoma cells as well as in normal pregnancy tissue.

The USA hCG Reference Service has seen 47 choriocarcinoma cases from around the world. All bloods were tested for the concentration of the hormone hCG and the autocrine hyperglycosylated hCG (Table 4). As found, the diagnoses made by the USA hCG Reference Service could be defined as Maximally Aggressive Choriocarcinoma (81%-100% hyperglycosylated hCG), Aggressive Choriocarcinoma (41-80% hyperglycosylated hCG), Minimally Aggressive Choriocarcinoma (1%-40% hyperglycosylated hCG), and Quiescent Choriocarcinoma (<1% hyperglycosylated hCG). Just based on the percent hyperglycosylated hCG (hyperglycosylated hCG/hyperglycosylated hCG + hormone hCG) the aggressiveness of choriocarcinoma can be accurately defined (Table 4) [2]. Interestingly, the percent hyperglycosylated hCG as a clinical parameter exactly correlated with the cancer doubling rate (weeks) as a parameter, $r^2 = 0.996$. As such, the measurement of percent hyperglycosylated hCG is an alternative measurement of cancer aggressiveness to, difficult to determine cancer doubling rate, that can be used by treating physicians.

Table 4. *Serum samples from 47 choriocarcinoma cases. hCG-H is the B152 hyperglycosylated hCG result in ng/ml, converted to hCG equivalents mIU/ml (X11) and the proportion hCG-H of total hCG (% hCG-H). The cancer doubling rate is the consulting physician's estimated time for the cancer mass to double.*

Age	Diagnosis made by USA hCG Reference Service	Total hCG (mIU/ml)	hCG-H (ng/ml)	hCG-H (mIU/ml)	Percent hCG-H hCG-H/hCG (%)	Cancer Doubling rate (weeks)
25	Maximally Aggressive Choriocarcinoma	40256	4400	48400	100%	2.6
32	Maximally Aggressive Choriocarcinoma	80400	8050	88550	100%	3
21	Maximally Aggressive Choriocarcinoma	314000	429000	390000	100%	<2
34	Maximally Aggressive Choriocarcinoma	399500	37270	401000	100%	<2
21	Maximally Aggressive Choriocarcinoma	932000	85090	936000	100%	<2
19	Maximally Aggressive Choriocarcinoma	50053	4333	47663	95%	Not determined
34	Maximally Aggressive Choriocarcinoma	116620	10011	110121	94%	<2
37	Maximally Aggressive Choriocarcinoma	596000	50931	560240	94%	5.5
N/A	Maximally Aggressive Choriocarcinoma	37500	3110	34210	91%	2.4
35	Maximally Aggressive Choriocarcinoma	141627	11034	121374	86%	<2
26	Maximally Aggressive Choriocarcinoma	45000	3400	37400	83%	<2
N/A	Maximally Aggressive Choriocarcinoma	40644	3012	33132	82%	2.6
			Mean ± SD		94% ± 6.9%	2.57 ± 1.09
N/A	Aggressive choriocarcinoma	6016	436	4796	80%	3.5
20	Aggressive choriocarcinoma	821	58	638	78%	4.2
N/A	Aggressive choriocarcinoma	2500	176	1936	77%	<3
N/A	Aggressive choriocarcinoma	80699	5560	61160	76%	Not determined
N/A	Aggressive choriocarcinoma	2450	140	1540	63%	4
43	Aggressive choriocarcinoma	1208	66	726	60%	4.4
34	Aggressive choriocarcinoma	901	49	539	60%	4

37	Aggressive choriocarcinoma	21590	982	10802	50%	3.5
29	Aggressive choriocarcinoma	454	19.2	211.2	47%	2.8
36	Aggressive choriocarcinoma	521	20.2	222.2	43%	3.5
34	Aggressive choriocarcinoma	2362	91	1001	42%	3.8
			Mean ± SD		60% ± 13%	3.85 ± 0.58
37	Minimally Aggressive choriocarcinoma	27688	982	10802	39%	5.5
27	Minimally Aggressive choriocarcinoma	440	15.1	166.1	38%	5.2
42	Minimally Aggressive choriocarcinoma	542	17	187	35%	4.5
50	Minimally Aggressive choriocarcinoma	1596	42	462	29%	Not determined
29	Minimally Aggressive choriocarcinoma	214	5.6	61.6	29%	5.5
32	Minimally Aggressive choriocarcinoma	5290	112	1232	23%	4
30	Minimally Aggressive choriocarcinoma	20440	4025	44275	22%	5
31	Minimally Aggressive choriocarcinoma	639	11.7	128.7	20%	5.5
37	Minimally Aggressive choriocarcinoma	735	7.8	85.8	12%	>6
46	Minimally Aggressive choriocarcinoma	238	2	22	9.2%	>6
			Mean ± SD		26 ± 10%	5.20 ± 0.66
46	Quiescent choriocarcinoma	18	0.01	0.11	0.61%	5.5
26	Quiescent choriocarcinoma	3.4	Not detected		<1%	>6
24	Quiescent choriocarcinoma	7.2	Not detected		<1%	>6
17	Quiescent choriocarcinoma	11	Not detected		<1%	>6
23	Quiescent choriocarcinoma	7.8	Not detected		<1%	>6
27	Quiescent choriocarcinoma	3.3	Not detected		<1%	>6
32	Quiescent choriocarcinoma	17	Not detected		<1%	>6
35	Quiescent choriocarcinoma	20	Not detected		<1%	>6
			Mean ± SD		<1 ± 0.013%	5.93 ± 0.17
36	Recurrent quiescent choriocarcinoma	28	1.3	14.3	51%	Not determined
55	Recurrent quiescent choriocarcinoma	22	1	11	50.0%	3.5
41	Recurrent quiescent choriocarcinoma	73	0.92	10.12	13.8%	Not determined
38	Recurrent quiescent choriocarcinoma	66	0.75	8.25	12.5%	4.5
19	Recurrent quiescent choriocarcinoma	224	2	19	8.5%	5
			Mean ± SD		26 ± 21%	4.37 ± 0.63
			T test		P=0.996	

It is concluded that in choriocarcinoma, exactly like pregnancy [1,2], that cancer invasion and growth is controlled by hyperglycosylated hCG, which promotes growth and invasion, and the hormone hCG, which attenuates growth and invasion. Percent hyperglycosylated hCG is an alternative measurement of cancer aggressiveness, to cancer doubling rate, that can be used with choriocarcinoma cases.

I considered other cancers, do they respond to the hormone hCG? As shown in Table 2, NTERA testicular germ cell cancer was promoted to grow by +18% and +32% by hyperglycosylated hCG, and diminished -67%, -70% and -70% by the hormone hCG. ScaBER bladder cancer was promoted to grow by +50% and +56% by hyperglycosylated hCG, and diminished -19%, -52% and -60% by the hormone hCG. T24 epithelial bladder cancer was promoted to grow by +10% and +29% by hyperglycosylated hCG, and diminished -15%, -22% and -66% by the hormone hCG. CaSki epidermoid cervical cancer was promoted to grow by +17% and +32% by hyperglycosylated hCG, and diminished -12%, -21% and -53% by the hormone hCG. KLE endometrial aneuploid cancer was promoted to grow by +17% and +32% by hyperglycosylated

hCG, and diminished -25%, -26% and -34% by the hormone hCG. HEC-1-a endometrial adenocarcinoma was promoted to grow by +38% and +66% by hyperglycosylated hCG, and diminished -9%, -39% and -55% by the hormone hCG.

Overall, cancers were promoted to grow by hyperglycosylated hCG +10% to +66%, and were declined by the hormone hCG -9% to -70%. Hyperglycosylated hCG promotes cell growth in pregnancy, choriocarcinoma and cancer, and the hormone hCG attenuates or blocks growth in pregnancy, choriocarcinoma and cancer.

Discussion

Very clearly, hyperglycosylated hCG promotes cell growth and cell invasion in pregnancy, choriocarcinoma and cancer cases. Hyperglycosylated hCG promotes malignancy [12]. The hormone hCG attenuates or blocks cell invasion and cell growth in pregnancy, choriocarcinoma and cancer cases.

It is inferred that the hCG/LH receptor is active in pregnancy, choriocarcinoma and cancer cases. It is probable, that when cells gather the hCG β-subunit gene as in carcinogenesis or transformation that the hCG/LH receptor gene may be directly connected, or turned on. Thus all cancer cells, just as they steal hCG β-subunit for malignancy [12], may get the hCG/LH receptor gene and thus respond to hCG.

Just as the hormone hCG attenuates or controls hyperglycosylated hCG-led invasion and growth in pregnancy, it may be used to attenuate or control malignancy in cancer patients, to control cancer while awaiting appropriate therapy or to suppress cancer in advanced cancer patients.

The hormone hCG has very little side effects in the post-menopausal women, and only promotes sexuality or testosterone production in the man. The hormone hCG might be administered intra-muscularly twice weekly to the patient with terminal cancer, to keep the cancer appropriately suppressed, and to achieve longevity with terminal cancer. Proportion hyperglycosylated hCG (hyperglycosylated hCG/ hyperglycosylated hCG+hormone hCG) might be useful to measure cancer aggressiveness status in choriocarcinoma cases.

Conflicts of Interest

The author declares that there are no conflicts of interest.

References

1. Elliott MM, Kardana A, Cole LA. Carbohydrate and Peptide structure of the α- and β-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. Endocrine. 1997; 7:15-32.
2. Cole LA. hCG and Hyperglycosylated hCG Carbohydrate Structures Corrected. J Glycobiol. 2014; 3:1000114.
3. Puett D, Bhowmick N, Lizelle M, et al. hCG-receptor binding and transmembrane signaling. Melec Cellul Emdocrinol. 1996; 125: 55-64.
4. Cole LA, Butler SA. The hCG/LH hormone receptor. In: Human chorionic gonadotropin (hCG) eds. Cole LA, Butler SA. Elsevier 2010.
5. Butler SA, Ikram MS, Mathieu S, et al. The increase in bladder carcinoma cell population induced by the free beta subunit of hCG is a result of an anti-apoptosis effect and not cell proliferation. Brit J Cancer. 2000; 82:1553-1556.
6. Berndt S, Blacher S, Munuat C, et al. Hyperglycosylated human chorionic gonadotropin stimulates angiogenesis through TGF-β receptor activation FASEB J. 2013; 12:213686.
7. Ahmud F, Ghosh S, Sinha S, et al. TGF-β-induced hCG-β regulates redox homeostasis in glioma cells Molec Cellul Biochem. 2015; 399:105-112.
8. Guibourdenche J, Handschuh K, Tsatsaris V, et al. Hyperglycosylated hCG is a marker of early human trophoblast invasion. J Clin Endocrinol Metab. 2010; 95:E240-E244.
9. Evans J. Hyperglycosylated hCG: a unique implantation and invasion factor. Euro J Immunol. 2016; 75:333-340.
10. Cole LA, Dai D, Leslie KK, et al. Gestational trophoblastic diseases: 1. Pathophysiology of hyperglycosylated hCG-regulated neoplasia. Gynecologic Oncology. 2006; 102:144-149.
11. Cole LA. Minimally-Aggressive Gestational Trophoblastic Neoplasms. Gynecol Oncol. 2012; 126:145-150.
12. Cole LA. Hyperglycosylated hCG drives malignancy in cancer cases. J Molec Oncol Res. 2017; 1:53-63.
13. Akhurst RJ, Balmain A. Genetic events and the role of TGF beta in epithelial tumour progression. J Pathol. 1999;187:82-90.
14. Murphy G, Reynolds JJ, Whitham SE, et al. Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. Euro Molec Biol Org J. 1987; 6:1899-1904.
15. Akhurst RJ. TGF-β antagonists: Why suppress a tumor suppressor? J Clin Invest. 2002; 109:533-1536.
16. Kliman HJ, Nestler JE, Sermasi E, et al. Purification, characterization, and in vivo differentiation of cytotrophoblasts from human term placentae. Endocrinology. 1986; 118: 1567-1582.
17. Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. N Engl J Med. 1999; 340:1796-1799.

*Correspondence to

Laurence A. Cole

USA hCG Reference Service,

Angel Fire, NM 87710,

USA

E-mail: larry@hcglab.com