CHAPTER

30

Cancer hCG, hyperglycosylated hCG, extravillous cytotrophoblastic hCG and TGFβ receptor 2

One gonadotropin, extravillous cytotrophoblast hCG, is an apparent centerpiece of cancer. It is in fact stolen by cancers, from pregnancy, to drive malignancy. Extravillous cytotrophoblast hCG emerges from human evolution as a super acidic, super potent TGFß agonists [1–3]. Super acidic and super potent as a result of the evolution of an increasingly acidic hCG pathway (see Chapter 2) [1–3].

The story of hCG and cancer is a personal one and much of the research described below was performed in my own research groups or with collaborators [4–6]. It started by looking at hCG as a tumor marker testing a total of 2,508 non-trophoblastic cancers (Including: bladder cancer, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, gastric cancer, hepatic cancer, lung cancer, intestinal cancer, lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, uterine cancer and vulvar cancer) in first morning urine samples. We also studied 91 trophoblastic cancers (choriocarcinoma, ovarian germ cell cancer and testicular germ cell cancer) using first morning urine samples and the B204 beta core fragment and nicked free ß-subunit assay (Table 30.1).

The 2,167 non-trophoblastic cancer patient urines and 91 trophoblastic cancer patient urine were tested in the B204 assay, with a 3.0 pmol/ml cancer cut-off. All 91 trophoblastic cancers were positive, 100% detection, yet only 949 of 2,167 non-trophoblastic cancer urines were positive, 44% detection (Table 30.1). It was concluded that a more sensitive B204 assay was needed for non-trophoblastic cancers.

A more sensitive assay was generated using ^{125}I-tracer antibody and 1/12,000 rather than 1/4,000 capture antibody. The more sensitive assay had a cut-off for cancer of 0.1 pmol/ml. Using this assay, all of 341 non-trophoblastic cancers were positive, 100% detection. It was concluded that trophoblastic cancers produce larger amounts of detectable material, 3.5-2,398,000 pmol/ml or 1.3–880,000 mIU/ml, and that non-trophoblastic cancers produce tiny concentrations of detectable material, 0.2-340 pmol/ml or 0.074-124 mIU/ml. We also concluded that all cancers produce one form of hCG or another.

But what form of hCG do cancers produce? Different hCG varients were measured using different assays: total hCG using the Siemens Immulite 1000 assay,

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Table 30.1 Urine B204 assay (cancer cut-off 3.0 pmol/ml) and super-sensitive B204 assay (cancer cut-off 0.1 pmol/ml), detects ß-core fragment, free ß-subunit, hyperglycosylated hCG free ß-subunit and nicked free ß-subunit.

	Urine B204 assay			**Urine B204 assay**		
Source	**Ultra-sensitive assay, Cut-off > 0.1 pmol/ml**			**Regular assay, Cut-off >3 pmol/ml**		
	#Cases	**#Detected**	**Sensitivity**	**#Cases**	**#Positive**	**Sensitivity**
A. Trophoblastic malignancies						
Choriocarcinoma				63	63	100%
Ovarian germ cell cancer				11	11	100%
Testicular germ cell cancer				17	17	100%
Total				**91**	**91**	**100%**
B. Non-trophoblastic malignancy						
Bladder cancer				140	62	44%
Breast cancer	130	130	100%	456	156	34%
Cervical cancer				410	197	48%
Colorectal cancer				80	29	36%
Endometrial cancer				233	103	44%
Gastric cancer				205	90	44%
Hepatic cancer				46	21	44%
Lung cancer	115	115	100%	154	38	25%
Intestinal cancer				17	8	47%
Lymphoma				41	13	32%
Ovarian cancer	56	56	100%	207	145	70%
Pancreatic cancer	40	40	100%	29	16	55%
Prostate cancer				12	9	75%
Renal cancer				66	32	48%
Uterine cancer				63	26	41%
Vulvar cancer				8	4	50%
Total	**341**	**341**	**100%**	**2167**	**949**	**44%**

C. Healthy						
NED, post cancer chemotherapy				33	2	6%
NED, post cancer surgery				21	1	5%
Healthy female, no cancer history				72	2	3%
Healthy male, no cancer history				28	1	4%
Total				**154**	**6**	**4%**
D. Benign Disease						
Benign gynecological lesion, tumor				28	0	0%
Follicular ovarian cyst, benign				97	1	1%
Cervical carcinoma in-situ				12	0	0%
Cervical dyskaryosis				66	2	3%
Condyloma				30	0	0%
Endometriosis				16	1	0%
Myoma				27	3	11%
Total				**276**	**7**	**2.5%**

hyperglycosylated hCG and extravillous cytotrophoblast hCG using the B152 assay, and all free ß-subunit using the FBT11 assay (Table 30.2).

Table 30.2 Content of cancer patient serum, measured using Immulite total hCG assay, B152 hyperglycosylated molecule assay, and FBT11 free ß-subunit assay.

Case or sample	Immulite 1000 assay (Total hCG)	B152 assay (Type 2 O-linked sugars)	B152 assay	FBT11 assay (Free ß-subunit)	FBT11 assay
	ng/ml	ng/ml	%	ng/ml	%
Standards					
Recombinant hCG (Ovidrel), 1000 ng/ml	1007	0.13	0.013%	1.17	0%
Recombinant hCG ß-subunit, 600 ng/ml	602	0.11	0.011%	615	102%
Hyperglycosylated hCG standard C5, 1000 ng/ml	997	995	100%	14	1%
C5 free ß-subunit standard, 600 ng/ml	578	687	100%	610	106%
Extravillous trophoblast hCG C5. 1000 ng/ml	1003	998	100%	5	1%
Trophoblastic cancers					
Choriocarcinoma	21.200	23.300	110%	1.172	6%
Choriocarcinoma	33.700	34.700	103%	1.610	5%
Choriocarcinoma	57.400	54.600	95%	5.680	10%
Choriocarcinoma	20.600	19.900	97%	2.010	10%
Choriocarcinoma	27.500	30.100	109%	5.330	19%
Choriocarcinoma	153.400	155.000	101%	15.740	10%
Choriocarcinoma	34.700	36.800	106%	1.230	4%
Choriocarcinoma	43.400	47.700	110%	1.250	3%
Choriocarcinoma	210.500	210.200	100%	15.750	7%
Choriocarcinoma	2.500	2.100	84%	155	6%
Choriocarcinoma	3.700	2.900	78%	505	14%
Choriocarcinoma	1.600	870	54%	360	23%
Choriocarcinoma	9.900	9.900	100%	1.260	13%
Choriocarcinoma	20.400	19.000	93%	1.880	9%
Choriocarcinoma	1.600	1.010	63%	29	2%
Choriocarcinoma	1.100	1.010	91%	106	10%
Choriocarcinoma	3.300	2.500	76%	230	7%
Choriocarcinoma	3.600	3.100	86%	410	11%
Choriocarcinoma	1.050	1.050	100%	89	8%

Table 30.2 Content of cancer patient serum, measured using Immulite total hCG assay, B152 hyperglycosylated molecule assay, and FBT11 free ß-subunit assay. (*Cont.*)

Case or sample	Immulite 1000 assay (Total hCG)	B152 assay (Type 2 O-linked sugars)	B152 assay	FBT11 assay (Free ß-subunit)	FBT11 assay
	ng/ml	ng/ml	%	ng/ml	%
Choriocarcinoma	6.300	5.900	94%	311	5%
Ovarian germ cell cancer	13.400	14.000	104%	2.230	17%
Ovarian germ cell cancer	8.800	8.800	100%	547	6%
Ovarian germ cell cancer	17.600	17.100	97%	3.120	18%
Ovarian germ cell cancer	33.400	34.300	102%	11.560	35%
Ovarian germ cell cancer	18.900	18.700	99%	196	1%
Ovarian germ cell cancer	16.200	16.100	99%	151	1%
Testicular germ cell cancer	10.400	10.600	102%	655	6%
Testicular germ cell cancer	14.500	14.700	99%	907	6%
Testicular germ cell cancer	19.100	19.300	101%	430	2%
Testicular germ cell cancer	11.000	11.100	101%	1.220	11%
Testicular germ cell cancer	9.200	9.500	103%	103	1%
Testicular germ cell cancer	13.700	14.300	104%	2.240	16%
Mean ± standard deviation		96 ± 12.6%		9.4 ± 7.3%	
Non-trophoblastic cancers					
Bladder cancer	0.09	0.09	100%	0.10	111%
Bladder cancer	0.09	0.10	111%	0.09	100%
Bladder cancer	0.12	0.12	100%	0.11	92%
Bladder cancer	0.15	0.15	100%	0.15	100%
Bladder cancer	1.3	1.4	108%	1.4	108%
Breast cancer	1.3	1.3	100%	1.2	92%
Breast cancer	0.09	0.10	111%	0.10	111%
Breast cancer	0.11	0.11	100%	0.12	109%
Breast cancer	0.66	0.62	94%	0.71	108%
Breast cancer	0.11	0.12	109%	0.11	100%
Breast cancer	0.14	0.14	100%	0.15	107%
Breast cancer	0.11	0.10	91%	0.10	91%
Breast cancer	0.09	0.09	100%	0.10	111%
Breast cancer	0.12	0.13	108%	0.10	83%
Breast cancer	1.1	1.0	91%	1.1	100%
Breast cancer	0.55	0.55	100%	0.50	91%
			100%		
Breast cancer	0.20	0.20	100%	0.18	90%
Breast cancer	0.10	0.08	80%	0.09	113%
Cervical cancer	0.13	0.13	100%	0.13	100%

(*Continued*)

Table 30.2 Content of cancer patient serum, measured using Immulite total hCG assay, B152 hyperglycosylated molecule assay, and FBT11 free ß-subunit assay. (*Cont.*)

Case or sample	Immulite 1000 assay (Total hCG)	B152 assay (Type 2 O-linked sugars)	B152 assay	FBT11 assay (Free ß-subunit)	FBT11 assay
	ng/ml	ng/ml	%	ng/ml	%
Cervical cancer	0.17	0.18	106%	0.18	106%
Cervical cancer	0.14	0.15	107%	0.14	100%
Cervical cancer	0.18	0.19	106%	0.18	100%
Cervical cancer	0.17	0.16	94%	0.17	100%
Cervical cancer	0.34	0.36	106%	0.35	103%
Cervical cancer	0.18	0.18	100%	0.20	111%
Cervical cancer	0.44	0.46	105%	0.40	91%
Cervical cancer	0.18	0.19	106%	0.18	100%
Cervical cancer	0.14	0.16	114%	0.12	86%
Endometrial cancer	0.16	0.16	100%	0.14	88%
Endometrial cancer	0.36	0.38	106%	0.32	89%
Endometrial cancer	0.09	0.09	100%	0.10	111%
Lung cancer	1.3	1.4	108%	1.4	108%
Lung cancer	0.70	0.75	107%	0.75	107%
Lung cancer	0.52	0.56	108%	0.51	98%
Lung cancer	0.32	0.36	113%	0.30	94%
Lung cancer	0.28	0.30	107%	0.31	111%
Lung cancer	0.11	0.11	100%	0.11	100%
Lung cancer	0.09	0.09	100%	0.10	111%
Ovarian cancer	0.23	0.24	104%	0.23	100%
Ovarian cancer	0.14	0.13	93%	0.15	107%
Ovarian cancer	0.11	0.10	91%	0.10	91%
Ovarian cancer	0.10	0.11	110%	0.10	100%
Ovarian cancer	0.10	0.09	90%	0.08	80%
Ovarian cancer	1.2	1.1	92%	1.0	83%
Ovarian cancer	0.62	0.65	95%	0.62	100%
Ovarian cancer	0.42	0.44	105%	0.44	105%
Ovarian cancer	0.21	0.21	100%	0.21	100%
Vulvar cancer	0.27	0.28	104%	0.23	85%
Vulvar cancer	0.13	0.13	100%	0.12	92%
Vulvar cancer	0.88	0.89	101%	0.85	97%
Vulvar cancer	0.67	0.70	104%	0.67	100%
Vulvar cancer	0.13	0.14	108%	0.12	92%
Mean ± standard deviation		102 ± 6.5%		99 ± 8.8%	

As shown in Table 30.2, 51 of 51 non-trophoblastic neoplasms serum samples, 100%, were positive in the Siemens Immulite assay, in the B152 (102 ± 6.5% of total hCG) and in the FBT11 assay (99 ± 8.8% of total hCG). This indicated that 51 of 51 cases were producing hyperglycosylated hCG or extravillous cytotrophoblast hCG free ß-subunit. Concanavalin A-Sepharose affinity chromatography (not shown) showed that the free ß-subunit had triantennary oligosaccharides or were extravillous cytotrophoblast free ß-subunit. Similarly, the total hCG and B152 immunoassays and the Concanavalin A-Sepharose test (not shown) indicated that 32 of 32 trophoblastic cancers were producing extravillous cytotrophoblast hCG. It was concluded that trophoblastic cancer only (100%, no exceptions seen) produce extravillous cytotrophoblast hCG dimer, and that non-trophoblastic cancer only (100%, no exceptions seen) produce extravillous cytotrophoblast hCG free ß-subunit.

Why were all non-trophoblastic cancers producing only beta subunit of hCG but all trophoblastic cancers were producing extravillous cytotrophoblast hCG? This question is best answered by a paper by Beebe et al [7]. In pregnancy, ß-subunit of hCG receives 6 disulfide bridges, and α-subunit of hCG receives 5 disulfide bridges. The last two disulfide bridges on ß-subunit, ß93-100 and ß26-110 are completed by a placental enzyme, placental disulfide isomerase. If hCG is produced by another cell other than a trophoblast cell, this enzyme is not present, and the disulfide bridges are not made. Without these disulfide bridges the ß-subunit just forms a free ß-subunit and does not combine with α-subunit to form αß dimer [7]. This was seemingly the case with cancer free ß-subunit.

Other authors have shown that the hCG molecules produced by cancer cells drove malignancy-like functions in cancer cells [8–15] (Table 30.3). Based on the studies described above, this must be extravillous cytotrophoblast hCG or its free ß-subunit. In 1996 Gillott et al. [8] showed that the ß-subunit produced by T24, ScaBER and RT112 bladder cancer cell line drove cancer cell line growth and blocked apoptosis in. In 2000 this was confirmed by Butler et al. [9] using ScaBER bladder cancer cell line and its ß-subunit. In 2002 Devi et al. [10] tested DU145 prostate carcinoma cell line and showed that the ß-subunit drove cancer cell line growth. Then, in 2006 our laboratory [11] used Jar and JEG-III choriocarcinoma cell line and showed that invasive cytotrophoblast hCG drove cancer growth and drove cancer cell invasion of other. Then, in 2008 Jankowska et al. [12] showed with tissue from 12 patients with planoepithelial cervical cancer, with one patient with glossy cell cervical cancer, one patient with basaloid cell cervical cancer, one patient with intraepitheliate cervical cancer, and 15 patients with endometrial cancer that the ß-subunit blocked apoptosis. In 2008 Li et al. [13] showed with tissue from 81 patients with uterine cervical cancer that the ß-subunit blocked apoptosis and in 2011 Guo et al. [14] looked at T29 and T80 epithelial ovarian cancer cell lines and tissue from 15 patient with ovarian carcinoma and showed that the ß-subunit produced by the cancers promoted cell growth and blocked apoptosis. Finally, and most recently, Kawamata et al. [15] examined tissue from 80 patients with colorectal cancer, examined Caco-2, LoVo, HCA-7, WiDr and T84 colorectal cancer cell lines and showed with them all that ß-subunit

Table 30.3 Eight independent reports that hyperglycosylated hCG and hCG ß-subunit promotes cancer cell malignancy (10-17).

Year	Author	Cancer cell tested	Promotes cancer cell growth	Promotes cancer cell invasion	Blocks cancer cell apoptosis	Ref
1996	Gillott et al.	T24 Epithelial bladder cancer cell line	X		X	5
		ScaBER squamous bladder cell line	X		X	
		RT112 bladder carcinoma cell line	X		X	
		5637 adherent bladder carcinoma cell line	X		X	
2000	Butler et al.	ScaBER squamous bladder cell line	X		X	6
2002	Devi et al.	DU145 prostate carcinoma cells	X			7
2006	Cole et al.	Jar choriocarcinoma cell line	X	X		8
		JEG-III choriocarcinoma cell line	X	X		
2008	Jankowska et al.	12 patients planoepithelial cervical cancer			X	9
		1 patient with glossy cell cervical cancer			X	
		1 patient with Basaloid cell cervical cancer			X	
		1 patient intraepitheliate cervical cancer			X	
		15 patients with endometrial cancer			X	
2008	Li et al.	81 patients with uterine cervical cancer			X	10
2011	Guo et al.	T29 ovarian epithelial carcinoma cell line	X		X	11
		T80 ovarian epithelial carcinoma cell line	X		X	
		15 patients with ovarian carcinoma	X		X	
2018	Kawamata et al.	80 patients with colorectal cancer	X	X		12
		Caco-2 epithelial colorectal cancer	X	X		
		LoVo epithelial colorectal cancer	X	X		
		HCA-7 epithelial colorectal cancer	X	X		
		WiDr colorectal adenocarcinoma	X	X		
		T84 epithelial colorectal cancer	X	X		

drove cancer growth and drove cell-cell invasion of other tissues. All of these studies are summarized in Table 30.3.

We went onto examine 10 different cancer cell lines, from choriocarcinoma, testicular germ cell cancer. endometrial adenocarcinoma, squamous bladder cancer, epithelial bladder carcinoma, epithelial lung cancer, Hodgkin's lymphoma and cervical carcinoma (Table 30.4). Again all 10 cancers produced extravillous cytotrophoblast hCG and its free ß-subunit and vastly cancer cell growth. It was concluded from these results (Tables 30.3 and 30.4), that extravillous cytotrophoblast hCG and its free ß-subunit seemingly drove malignancy in all or most cancers.

After the development of monoclonal antibody B152 (a monoclonal antibody raised against extravillous cytotrophoblast hCG and hyperglycosylated hCG and their free ß-subunit which did not bind the hormone hCG [16]) it was possible to immobilize extravillous cytotrophoblast hCG and its free ß-subunit as produced by cancer cell lines. As shown in Table 30.5 treatment with B152, 2.0 μg/ml, took the malignancy out of cancer so that it did not grow at all over 24 hours. Two conclusions were made. Firstly, this confirmed and proved that extravillous cytotrophoblast hCG and its free ß-subunit controlled malignancy in all or most cancers. Secondly, that this confirms that extravillous cytotrophoblast hCG and its free ß-subunit are the primary drivers of malignancy in cancer cells.

Furthermore eight nude mice were transplanted subcutaneously with JEG-III human choriocarcinoma cells and 8 nude mice were transplanted subcutaneously with Caski human cervical carcinoma cells. In each transplant 10 million cancer cells total were transplanted into 6 skin sites on nude mice. After two weeks, multiple metastases were present on the skin of animals, tumors as large as 2 cm in diameter in size. Athymic mice were then given either antibody B152 (2×4 mice) or control mouse immunoglobulin G (IgG) (2×4 mice), 0.3 mg intraperitoneally injected twice weekly for two weeks or until 4 weeks cancer time. The tumor cross-section area was measured weekly with calipers before every treatment according to the formula: length x width x π x 4. Termination was mandatory at 4 weeks for University of New Mexico Health Science Center Animal Resource Center.

As shown in Fig. 30.1, IgG did nothing but let both cancers continue to grow and metastasize to >300% of two weeks size. B152 immediately blocked the cancers, blocking all cancer malignancy. After 4 weeks the choriocarcinoma was if anything 76% the size of the cancer at 2 weeks cancer time, and the cervical cancer was if anything 91% of the size of the cancer at 2 weeks.

In conclusion, B152 antibody treatment eliminated the malignancy of the human choriocarcinoma (Fig. 30.2A) and of the human cervical carcinoma (Fig. 30.2B) in athymic mice, deactivated the cancer.

Extravillous cytotrophoblast hCG and its free ß-subunit blocks apoptosis in human cancer cases (Table 30.3) and blocks immune response to the cancer. If B152 was humanized by established DNA technology [17–19], B152 would possibly be an effective cure for cancer, blocking malignancy and without extravillous cytotrophoblast hCG or its free ß-subunit apoptosis and the immune system would destroy all remnant cancer tissue. Alternatively put, B152 could be a cure for all or most human

Table 30.4 Promotion of cancer cell 24 h growth at 70% confluency by C5 extravillous cytotrophoblast hCG. All experiments carried out in quadruplicate. Sixteen T75 flask of each cancer cell line grown to approximately 70% confluent. Four flasks rejected to prevent 70% confluence imbalance, 4 flask used for 70% confluence cell count. No additive flask results (-70% count) considered as blank and subtracted from all results. In case of Jar choriocarcinoma cells, for instance, mean no additive result 100,360 cells, mean 70% confluency result 128,880 cells, blank = 128880-100360 = 28,520 cells, blank = 100%. 20ng extravillous cytotrophoblast hCG mean result 158540 cells, 158540-blank = 130020 cells, 130020/100360 x 100 = 130%.

Cells	Supplement added to culture fluid	% effect on cell count	T test
Jar choriocarcinoma	No additive	100% cell count after 24 h	P = 0.0123
	C5 extravillous cytotrophoblast hCG 2ng/ml	112% cell count after 24 h	
	C5 extravillous cytotrophoblast hCG 20 ng/ml	130% cell count after 24 h	
JEGIII choriocarcinoma	No additive	100% cell count after 24 h	P = 0.0018
	C5 extravillous cytotrophoblast hCG 2 ng/ml	110% cell count after 24 h	
	C5 extravillous cytotrophoblast hCG hCG 20 ng/ml	128% cell count after 24 h	
NTERA Testicular germ cell	No additive	100% cell count after 24 h	P = 0.0018
	C5 extravillous cytotrophoblast hCG 2 ng/ml	118% cell count after 24 h	
	C5 extravillous cytotrophoblast hCG 20 ng/ml	132% cell count after 24 h	
Hec-1-a Endometrial adenocarcinoma	No additive	100% cell count after 24 h	P = 0.0021
	C5 extravillous cytotrophoblast hCGß 2 ng/ml	138% cell count after 24 h	
	C5 extravillous cytotrophoblast hCGß 20 ng/ml	166% cell count after 24h	
ScaBER squamous bladder cancer	No additive	100% cell count after 24 h	P = 0.010
	C5 extravillous cytotrophoblast hCGß 2 ng/ml	150% cell count after 24 h	
	C5 extravillous cytotrophoblast hCGß 20 ng/ml	156% cell count after 24h	

Table 30.4 Promotion of cancer cell 24 h growth at 70% confluency by C5 extravillous cytotrophoblast hCG. All experiments carried out in quadruplicate. Sixteen T75 flask of each cancer cell line grown to approximately 70% confluent. Four flasks rejected to prevent 70% confluence imbalance, 4 flask used for 70% confluence cell count. No additive flask results (-70% count) considered as blank and subtracted from all results. In case of Jar choriocarcinoma cells, for instance, mean no additive result 100,360 cells, mean 70% confluency result 128,880 cells, blank = 128880-100360 = 28,520 cells, blank = 100%. 20ng extravillous cytotrophoblast hCG mean result 158540 cells, 158540-blank = 130020 cells, 130020/100360 x 100 = 130%. (*Cont.*)

Cells	Supplement added to culture fluid	% effect on cell count	T test
KLE Endometrial adenocarcinoma	No additive	100% cell count after 24 h	P = 0.0.001
	C5 extravillous cytotrophoblast hCGß 2 ng/ml	117% cell count after 24 h	
	C5 extravillous cytotrophoblast hCGß 20 ng/ml	132% cell count after 24 h	
T24 epithelial bladder carcinoma	No additive	100% cell count after 24 h	P = 0.011
	C5 extravillous cytotrophoblast hCGß 2 ng/ml	137% cell count after 24 h	
	C5 extravillous cytotrophoblast hCGß 20 ng/ml	148% cell count after 24 h	
SK-MES-1 epithelial lung cancer	No additive	100% cell count after 24 h	P = 0.0001
	C5 extravillous cytotrophoblast hCGß 2 ng/ml	142% cell count after 24 h	
	C5 extravillous cytotrophoblast hCGß 20 ng/ml	172% cell count after 24 h	
KM-H2 Hodgkin's lymphoma	No additive	100% cell count after 24 h	P = 0.022
	C5 extravillous cytotrophoblast hCGß 2 ng/ml	121% cell count after 24 h	
	C5 extravillous cytotrophoblast hCGß 20 ng/ml	139% cell count after 24 h	
CaSki Cervical carcinoma	No additive	100% cell count after 24 h	P = 0.010
	C5 extravillous cytotrophoblast hCGß 2 ng/ml	132% cell count after 24 h	
	C5 extravillous cytotrophoblast hCGß 20 ng/ml	146% cell count after 24 h	

Table 30.5 Cancer cells cultured to 70% flask confluency, then cultured 24 h with antibody B152 in quadruplicate and cells counted. B152 is monoclonal antibody B152 Values are percent change from 70% confluency. The 70% confluency column lists the percentage cells and the cell count ± standard deviation, all other columns just list percentage cells ± standard deviation.

Cancer cell line	70% confluency	B152 0 μg/ml	B152 0.5 μg/ml	B152 1.0 μg/ml	B152 2.0 μg/ml
Jar choriocarcinoma	100 ± 10% 334,500 ± 33,000	128 ± 8.4%	110 ± 23%	105 ± 15%	101 ± 0.9%
JEG-III choriocarcinoma	100 ± 7.7% 430,000 ± 33,110	123 ± 11%	115 ± 7.6%	103 ± 2.4%	101 ± 8.1%
NTERA testicular germ cell	100 ± 2.6% 391,000 ± 10,160	114 ± 19%	119 ± 10%	117± 2.4%	100 ± 0.7%
ScaBER bladder cancer	100 ± 5.1% 334,000 ± 17,000	147 ± 2.0%	129 ± 16%	120 ± 3.7%	100 ± 2.9%
T24 bladder cancer	100 ± 4.2% 559.000 ± 24,000	120 ± 4.4%	114 ± 12%	103 ± 1.1%	100 ± 3.5%
Hec-1-a endometrial cancer	100 ± 3.3% 390,500 ± 12,800	140 ± 11%	128 ± 7.7%	132 ± 8.2%	103 ± 3.1%
KLE endometrial cancer	100 ± 2.1% 331,000 ± 6,950	182 ± 16%	142 ± 1.3%	118 ± 3.3%	102 ± 2.5%

cancers and removing these forms of hCG from the circulation could present a very interesting therapy for treating cancers that produce these molecules [20] and that specifc antibody therapies will need to take into consideration glycovariation on hCG to ensure effectiveness [21].

We know that the production of hCG is linked to the growth and invasion in cancer cells but what is the mode of action. Multiple authors have shown that the ß-subunit of hyperglycosylated hCG and extravillous cytotrophoblast hCG and free ß-subunit act on TGFß receptor 2. It is through this receptor that hCG can block apoptosis, promote cell growth and promote invasion by promoting metalloproteinases and promoting collagenases [9, 22, 23].

Interestingly, Butler and colleagues [9] who tested TGFß receptor 2 activity in bladder cancer cells claim that hCG free ß-subunit are antagonists and can be specifically competed out. Berndt and colleagues [22] used LHCGR mouse aortic ring cells and insist that it is an agonist. Ahmad and colleagues found that overall hCG ß-subunit formed a special previously unknown link with TGFß that changed everything. Whatever this previously unknown link may be, it allows hCG ß-subunit take control of the TGFß receptor.

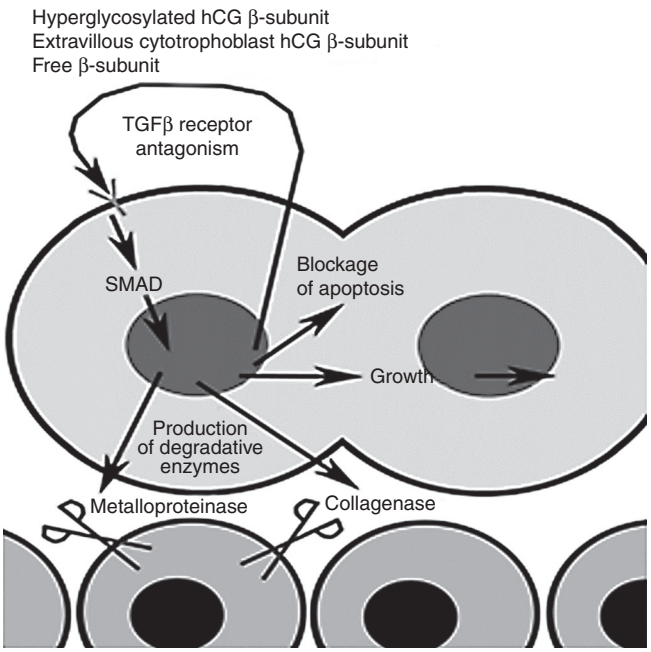

FIGURE 30.1

Action of hyperglycosylated hCG, hyperglycosylated hCG free β-subunit, and hCG free β-subunit on TGFβ receptor 2.

A model of hyperglycosylated hCG, hyperglycosylated hCG free β-subunit, or hCG free β-subunit antagonizing cytotrophoblast cells in blastocyst implantation or cancer malignancies is shown in Fig. 30.1. As illustrated, TGFβ-II receptors act through SMAD and cAMP intermediates, which permit nuclear penetration. SMADs have been demonstrated in the response of TGFβ receptor 2 to hyperglycosylated hCG [22]. Angiogenesis has also been demonstrated in response to hyperglycosylated hCG [23] and that hyperglycosylated hCG and hyperglycosylated hCG free β-subunit are interchangeable promoters with similar potency has also been demonstrated [24].

Lustabader et al., Lapthorn et al., and Wu et al. [25–28] have demonstrated a common cystine knot structure in hCG (on hCG α-subunit and β-subunit) linking the structure of hCG and TGFβ. That the hCG amino acid sequence with different glycosylation (hyperglycosylation) can bind the LH/hCG hormone receptor and the TGFβ-II autocrine receptor is rather unusual, giving the molecule two distinctly different functions (hCG and hyperglycosylated hCG) and two distinct sets of actions. These mechanisms and actions of hCGβ in epithelial cancer were proposed almost twenty years ago [29] have since been discussed in detail by Butler and collaborators [30,31].

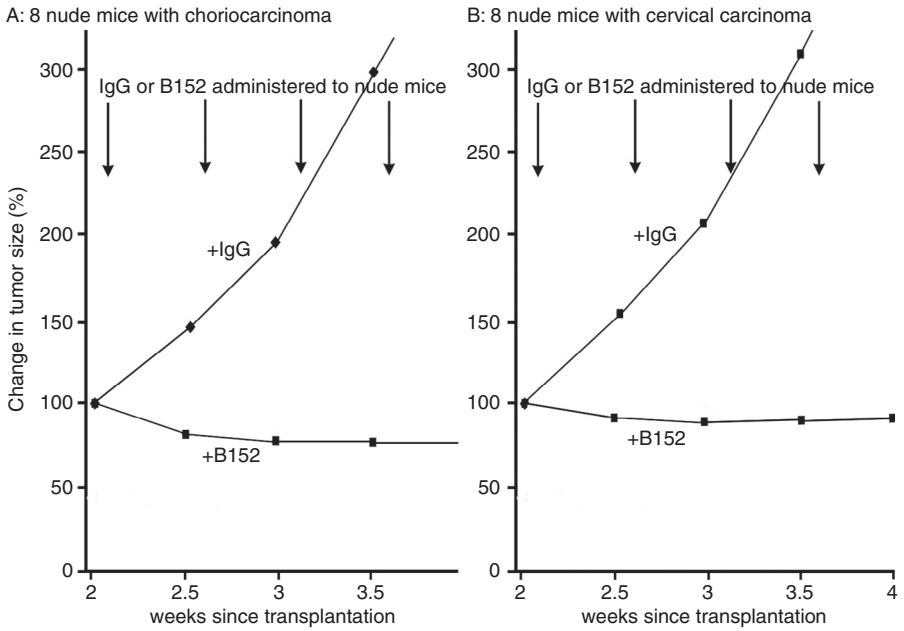

FIGURE 30.2

Eight nude mice transplanted with 10,000,000 cells JEG-III human choriocarcinoma cells (A), and 8 nude mice transplanted with 10,000,000 cells CaSki cervical carcinoma (B). 2 weeks later half of each 8 treated with antibody B152 and half treated with non-specific immunoglobulin G (IgG).

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