AUTHOR QUERY FORM

ELSEVIER

Journal: MCE

Article Number: 8093

Please e-mail or fax your responses and any corrections to:

E-mail: corrections.esch@elsevier.sps.co.in

Fax: +31 2048 52799

Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list. Note: if you opt to annotate the file with software other than Adobe Reader then please also highlight the appropriate place in the PDF file. To ensure fast publication of your paper please return your corrections within 48 hours.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Click on the 'Q' link to go to the location in the proof.

Location in article	Query / Remark: click on the Q link to go Please insert your reply or correction at the corresponding line in the proof
Q1	Please confirm that given names and surnames have been identified correctly.
Q2	Highlights are 3–5 bullet points, no more than 85 characters per bullet point. Please provide it in correct format. For more information, see www.elsevier.com/highlights.
Q3	Please check the insertion of keywords from metadata and correct if necessary.
Q4	Refs. "Gillott et al. (1996) and Cole et al. (2006c)" are cited in text but not listed. Please check.
Q5	This section comprises references that occur in the reference list but not in the body of the text. Please position each reference in the text or, alternatively, delete it. Any reference not dealt with will be retained in this section.
Q6	Please check all the authors names in the Ref. "Cole et al. (2004)".

Thank you for your assistance.

Highlights

▶ This article examines and shows that Hyperglycosylated hCG, Hyperglycosylated hCGβ and hCGβ are interchangeable in choriocarcinoma, bladder and endometrial cancer. ▶ Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ may all function at TGFβ antagonists. ▶ hCG has no inter-relatable activity. ▶ Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ are inter-relatable cancer promoters.

Molecular and Cellular Endocrinology xxx (2011) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ: Interchangeable cancer promoters

Laurence A. Cole[a,*], Stephen Butler[b]

[a] USA hCG Reference Service, Department of Obstetrics and Gynecology, Health Sciences Center MSC10-5580, 1 University of New Mexico, Albuquerque, NM 87104, USA
[b] Centre for Investigative and Diagnostic Oncology, Middlesex University, London NW4 4BT, UK

ARTICLE INFO

Article history:
Received 27 July 2011
Received in revised form 26 October 2011
Accepted 26 October 2011
Available online xxxx

Keywords:
hCG
Hyperglycosylated hCG
hCGβ

ABSTRACT

Introduction: Several groups are researching cancers, and showing that hCGβ is a promoter of cancer growth and malignancy. Recent research shows that some hCGβ is present as Hyperglycosylated hCGβ. Other groups studied Hyperglycosylated hCG as a promoter of choriocarcinoma and germ cell malignancies. The question therefore arises, are Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ interrelated or interchangable promoters of cancer?
Methods: The actions of Hyperglycosylated hCGβ, hCGβ and Hyperglycosylated hCG are investigated in 7 cell lines, Jar and JEG-3 choriocarcinoma cell lines, NTERA germ cell cancer line, SCaBER and T24 bladder epithelial carcinoma lines, KLE and Hec-1-a endometrial adenocarcinoma and epithelial carcinoma cell lines. Actions of promoters on cell growth are investigated.
Results: The actions of Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ appear to be interchangeable in all cell lines investigated.
Discussion: All hCG-related cancer promoters seem interrelated, working through a similar mechanism, antagonism of apoptosis through know receptors such as TGFβ receptors in all cancers studied.

1. Introduction

Numerous publications have shown that forms of chorionic gonadotropin (hCG) mark many human malignancies. These includes choriocarcinoma and germ cell malignancies, and other malignancies including most epithelial malignancies or carcinomas, adenocarcinomas, sarcomas, teratomas, blastomas, leukemias and lymphomas (Acevedo and Hartstock, 1996; Alfthan et al., 1992; Bellet et al., 1997; Butler et al., 2000; Butler and Iles, 2003, 2010; Cole et al., 1982, 2006a,b; Cole, 1994; Cole and Hussa, 1981; Cosgrove et al., 1989; Halila et al., 1989; Hussa et al., 1986; Iles 2007; Iversen et al., 2003; Li et al., 2008; Puisieux et al., 1990; Ruddon and Norton, 1993; Regelson, 1995).

It has been shown that choriocarcinoma and germ cell malignancies produce a large variant of hCG dimer (Cole, 1987; Cole et al., 2006a; Cole, 2007; Elliott et al., 1997; Martell and Ruddon, 1990; Ruddon and Norton, 1993). It has also been shown that these malignancies produce primarily Hyperglycosylated hCG (hCG-H), a form of hCG with larger carbohydrate side chains where the O-linked oligosaccharides are strikingly different between pregnancy and choriocarcinoma hCG (Cole, 1987; Elliott et al., 1997; Valmu et al., 2006). hCG-H has recently been shown to have autocrine activity promoting placental implantation in pregnancy, and growth and invasion by choriocarcinoma and germ cell cancer cells, contributing to malignancy. This makes hCG-H an autocrine cancer cell promoter rather than a simple "gonadotrophin" hormone acting on ovarian and pregnancy tissues (Cole et al., 2006a,b; Guibourdenche et al., 2010; Handschuh et al., 2007a,b).

As a general rule it is the free hCGβ-subunit (hCGβ) or a large variant of hCGβ (hCGβ-large) which marks all other malignancies, (Bellet et al., 1997; Butler et al., 2000; Butler and Iles, 2003, 2010; Cole and Hussa, 1981; Cole et al., 1982; Carter et al., 2006; Cosgrove et al., 1989; Halila et al., 1989; Hussa et al., 1986; Iles, 2007; Iversen et al., 2003; Li et al., 2008; Ruddon and Norton, 1993). hCGβ-large has recently been demonstrated to be a hyperglycosylated β-subunit (hCGβ-H), with a similar larger sugar structure to that of hCG-H (Valmu et al., 2006). Research shows that hCGβ and hCGβ-H, as produced by cancer cells, are also autocrine growth factors, directly promoting cancer cell invasion, cancer cell growth and metastases (Bellet et al., 1997; Gillott et al., 1996; Butler et al., 2000; Butler and Iles, 2003; Carter et al., 2006; Cosgrove et al., 1989; Iles, 2007). Vaccines have now been generated against hCGβ to block hCGβ and hCGβ-H in the treatment of cancer (Moulton et al., 2002; Iversen et al., 2003; Butler et al., 2003; Delves et al., 2007; Butler and Iles, 2010; Morse et al., 2011). The vaccines have led to significant affects being demonstrated in vitro (Butler et al., 2003; Morse et al., 2011) and improvement in cancer survival times in phase II studies (Moulton et al., 2002), indicating the key role that the hCGβ and hCGβ-H pathway may play in cancer promotion.

* Corresponding author. Tel.: +1 505 263 9635; fax: +1 505 272 3576.
E-mail address: larry@hcglab.com (L.A. Cole).

0303-7207/$ - see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2011.10.029

Please cite this article in press as: Cole, L.A., Butler, S. Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ: Interchangeable cancer promoters. Molecular and Cellular Endocrinology (2011), doi:10.1016/j.mce.2011.10.029

However, some uncertainty exists regarding hCGβ or hCGβ-H and all non-placental and non-germ cell cancers. Do these cells produce hCGβ or hCGβ-H, as indicated in recent carbohydrate structure study (Valmu et al., 2006). In this article, we address this issue. We develop for the first time a specific assay for detecting hCGβ only (anti-β capture with anti core hCG β-subunit tracer) only and secondly a specific assay for detecting hCGβ-H only (anti hCG-H/hCGβ-H capture with anti β tracer) and confront this dilemma. Research shows that hCG β-subunit has common evolutionary origins with TGFβ (transforming growth factor-β) (Laub and Jennissen, 2003; Lehnert and Ankhurst, 1988), and that the crystal structure of hCG contains a cystine knot with 4 peptides common to hCG β-subunit, TGFβ, PDFG (platelet derived growth) and NGF (nerve growth factor) unexposed in the heart of the hCG molecule (Lapthorn et al., 1994). It has been shown that hCGβ antagonizes the TGFβ receptor on cancer cells (Butler et al., 2000; Butler and Iles, 2010) which leads to cell growth and invasion and blockage of cellular apoptosis (Bellet et al., 1997; Butler et al., 2000; Butler and Iles, 2003, 2010; Carter et al., 2006; Cosgrove et al., 1989; Iles, 2007; Li et al., 2008).

An abundance of evidence suggests that hCG-H may directly promote growth and invasion of placental and germ cell malignancies by a similar TGFβ antagonism pathway. This evidence starts with multiple publications on the involvement of a TGFβ receptor in the placental implantation process and the choriocarcinoma invasion process (Fisher et al., 1989; Hwang et al., 2001; Kamijo et al., 1998; Kingsley-Kallesen et al., 1997; Knittel et al., 1999; Liu et al., 2005; Murphy et al., 1987; Pampferf 2000; Pringle and Roberts, 2007; Qureshi et al., 2005; Schuster and Krieglstein, 2002; Shooner et al., 2005; Staun-Ram and Shaleu, 2005; Stetler-Stevenson et al., 1990). It appears that research on hCG-H has proceeded independently to research on hCGβ and hCGβ-H, with no investigation of the possibility that the molecules are analogous or interchangeable. Here we investigate common or interchangeable biological features of hCG-H and hCGβ.

2. Methods

Total hCG was measured using the Diagnostics Products Corp. (Los Angeles CA) Immulite 1000 automated platform. As published, this assay detects hCG, hCG-H, hCGβ and hCGβ-H on equi-molar basis (Cole et al., 2004). The sensitivity of this assay is 0.0024 nmol/L. hCGβ was measured using the Immulite 1000 platform hCGβ test. This assay detects hCGβ, detection limit 0.001 nmol/L, hCGβ but not hCGβ-H. Intact hCG-H dimer was measured in the microtiter plate assay using B152 anti-hCG-H/hCGβ-H capture antibody with 4001 anti-α peroxidase tracer antibody (Birken et al., 1999). This assay, detection limit 0.001 nmol/L, detects only hCG-H dimer and nicked hCG-H dimer. hCGβ-H was measured in the assay using B152 anti-hCG-H/hCGβ-H capture antibody with B204 anti-β peroxidase tracer antibody. This assay, detection limit 0.001 nmol/L, detects only hCGβ-H and nicked hCGβ-H. The general methods used for microtiter plate assays have been described previously (Cole et al., 2010).

The hCGβ preparation used to promote cell growth was CR129 (National Institutes of Health, Bethesda, MD). As discovered, the hyperglycosylation structure of hCGβ-H is analogous to the β-subunit of hCG-H (Valmu et al., 2006). As such, we used dissociated choriocarcinoma C5 hCG-H β-subunit as hCGβ-H stimulant (C5β) (Elliott et al., 1997). We used choriocarcinoma C5 hCG-H as stimulant (Elliott et al., 1997). We used the Chinese hamster ovary cell (CHO cell) recombinant hCG for this experiment.

Seven cell lines were obtained from the American Type Culture Collection (ATCC) (Manassas, VA), Jar and JEG-3 choriocarcinoma cell lines, NTERA testicular germ cell carcinoma cell line, SCaBER and T24 bladder epithelial carcinoma cell lines, KLE endometrial adenocarcinoma cell line, and Hec-1-a endometrial epithelial carcinoma cell line. The USA hCG Reference Service occasionally diagnoses non-placental and non-germ cell cancer in women with elevated total hCG outside of pregnancy (Cole et al., 2008). This is diagnosed by finding that most of the total hCG immunoreactivity is due to hCGβ. We found that we were only able to detect hCGβ-H in those cases with total hCG > 0.20 nmol/L.

Seven serum samples were obtained from non-placental and non-germ cell cancer cases, pre-therapy, and tested for hCGβ and hCGβ-H. Nine serum samples were also collected from choriocarcinoma cases pre-therapy, and tested in these same assays. These were USA hCG Reference Service samples and patient tests. Use of this data was monitored by the University Human Research and Review Committee (HRRC #2-548).

All cell cultures were maintained in Dulbecco's modified Eagle's Medium with 10% fetal bovine serum Gibco, Grand Island NY. Cells were harvested with 1× trypsin and EDTA (Gibco). Cells were grown 2–4 days after harvesting to 70% flask confluency, as estimated under the inverted microscope. Cells were then cultured for 24 h in Dulbecco's modified Eagle's Medium in 10% fetal bovine serum T-25 flasks with 3 ml medium, in quadruplicate, with sterile (filter sterilized) hCG, hCGβ, hCG-H and hCGβ-H, 0–2.7 nmol/L. Cells were also grown in quadruplicate with filter sterilized antibody B152, 0–20 μg/ml. At the completion of the 24 h incubation cells were released from the flasks with 1× trypsin and EDTA and counted in 8 positions in a glass hemocytometer under an inverted microscope.

All data were stored in Microsoft Excel spreadsheets. Means of quadruplicate cell counts were determined, as were standard deviation. Student's *t*-test was determined using the program Stat Sak (Dallal GE, Malden, MA) to assess significance of findings.

3. Results

We assessed 70% confluent cell culture medium from 7 cell lines and non-placental non-germ cell cancer serum using specific assays for detecting total hCG. The assay used detects hCG, hCG-H, hCGβ and hCGβ-H on equimolar basis (Cole et al., 2004) (Table 1). As shown, the two choriocarcinoma cell lines (Jar and JEG-3) and the one germ cell malignancy cell line (NTERA) all produced predominantly hCG-H. Similarly the 9 pre-therapy choriocarcinoma serum samples all produced primarily hCG-H, as shown previously (Cole et al., 2006c). In contrast, the 4 non-placental, non-germ cell cancer cell lines all produced a widely varying mixture of hCGβ (3–80%) and hCGβ-H (20–94%). Similarly, the 7 pre-therapy non-placental non-germ cell malignancy serum samples (ovarian, cervical, colorectal, endometrial and lung carcinomas) produced widely varying mixtures of hCGβ (14–93%) and hCGβ-H (13–74%). It is concluded that while placental and germ cell malignancies produce primarily hCG-H, other malignancies produce a widely varying mixture of hCGβ and hCGβ-H.

As shown (Table 2), both hCGβ and hCGβ-H can stimulate growth of 4 of 4 non-placental non-germ cell malignancy cell lines suggesting that both can function similarly. hCGβ and hCGβ-H both promoted growth of 4/4 cell lines in a significant manner. hCGβ, 0.27 nmol/L, promoted the 4 cell lines, SCaBER, KLE, T-24 and Hec-1-a cell line, to grow to 133%, 131%, 155% and 122%, respectively. hCGβ-H, 0.27 nmol/L. promoted the 4 cell lines to grow to 124%, 135%, 117% and 118%, respectively. No significant difference is noted in the hCGβ and hCGβ-H actions by *t*-test ($P = 0.20$).

We examined the interchangeability of hCGβ and hCG-H (Table 3). As shown (Table 3), hCG-H, 0.27 nmol/L, promotes Jar and JEG-3 choriocarcinoma cell lines to grow to 127% and 113% respectively. hCGβ promoted these same cell lines to grow to 148% and 116%. As shown, hCGβ, 0.27 nmol/L promotes the 4 non-placental non-germ cell cancer cell lines to grow to 133%,

Please cite this article in press as: Cole, L.A., Butler, S. Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ: Interchangeable cancer promoters. Molecular and Cellular Endocrinology (2011), doi:10.1016/j.mce.2011.10.029

Table 1
Characteristics of 70% confluent T25 cell culture medium and non-placental malignancies using total hCG assay, hCGβ assay, hCG-H assays, and hCGβ-H assays. Percentages are relative to total hCG.

	Total hCG assay (nmol/L)	β (nmol/L)	hCG-H (nmol/L)	hCGβ-H (nmol/L)
JArchoriocarcinoma cell line	5.9	0.90 (15%)	4.11 (70%)	0.91 (15%)
JEG-3 choriocarcinoma cell line	5.2	<0.0010	5.2 (100%)	<0.001
NTERA testicular germ cell carcinoma cell line	0.011	0.0041 (39%)	0.0065 (61%)	<0.001
SCaBERbladder epithelial carcinoma cell line	0.21	0.17 (80%)	<0.0010	0.043 (20%)
KLE endometrial adenocarcinoma cell line	0.053	0.0027 (5%)	<0.0010	0.050 (94%)
T24 bladder epithelial carcinoma cell line	0.038	0.0010 (3%)	<0.0010	0.037 (97%)
Hec-1-a endometrial epithelial carcinoma cell line	0.19	0.086 (44%)	<0.0010	0.11 (56%)
Cancer serum, non-placental 1	0.73	0.38 (52%)	<0.0010	0.35 (48%)
Cancer serum, non-placental 2	0.86	0.12 (14%)	<0.0010	0.74 (86%)
Cancer serum, non-placental 3	0.20	0.05 (26%)	<0.0010	0.14 (74%)
Cancer serum, non-placental 4	0.47	0.30 (64%)	<0.0010	<0.005
Cancer serum, non-placental 5	0.71	0.41 (58%)	<0.0010	0.23 (32%)
Cancer serum, non-placental 6	0.58	0.54 (93%)	<0.0010	<0.005
Cancer serum, non-placental 7	0.94	0.82 (87%)	<0.0010	0.12 (13%)
Choriocarcinoma serum 1	147	37 (26%)	197 (>100%)	<0.01
Choriocarcinoma serum 2	1477	38 (2.6%)	1298 (88%)	<0.01
Choriocarcinoma serum 3	7907	484 (6.1%)	6374 (81%)	<0.01
Choriocarcinoma serum 4	2095	173 (8.0%)	2426 (>100%)	<0.01
Choriocarcinoma serum 5	93	34 (36%)	111 (>100%)	<0.01
Choriocarcinoma serum 5	111	20 (18%)	137 (>100%)	<0.01
Choriocarcinoma serum 7	148	38 (26%)	198 (>100%)	<0.01
Choriocarcinoma serum 8	289	45 (15%)	300 (>100%)	<0.01
Choriocarcinoma serum 9	15	0.072 (0.5%)	12 (80%)	<0.01

Table 2
The action of hCGβ and hCGβ-H on growth of SCaBER, T24, KLE and Hec-1-a cells. Cells were cultured in T-25 flasks to 70% flask confluency (24 flasks per cell line, 6 condition in quadruplicate) and then cultured 24 h with the addition of hCGβ, 0, 0.027 and 0.27 nmol/L and hCG-Hβ, 0, 0.027 and 0.27 nmol/L. All flasks were then cell counted in quadruplicate. Results are mean counts (× 1000) ± standard deviation.

Cell line and additives	Cell count after 24 h culture (× 1000)			*t*-Test, 0.27 nmol/L vs.0 nmol/L
	0 nmol/L	0.027 nmol/L	0.27 nmol/L	
1. SCaBER bladder epithelial carcinoma cell line				
With hCGβ added	366 ± 18 (100%)	441 ± 41 (120%)	488 ± 33 (133%)	$P = 0.0049$
With hCGβ-H added	366 ± 18 (100%)	419 ± 2.0 (114%)	455 ± 14 (124%)	$P = 0.0028$
2. KLE endometrial adenocarcinoma cell line				
With hCGβ added	417 ± 7.1 (100%)	510 ± 7.2 (122%)	547 ± 14 (131%)	$P = 0.0001$
With hCGβ-H added	417 ± 7.1 (100%)	492 ± 11 (118%)	565 ± 13 (135%)	$P = <0.0002$
3. T24 bladder epithelial carcinoma cell line				
With hCGβ added	708 ± 12 (100%)	842 ± 48 (119%)	1095 ± 34 (155%)	$P = <0.00005$
With hCGβ-H added	1077 ± 25 (100%)	1165 ± 82 (108%)	1262 ± 27 (117%)	$P = 0010$
4. Hec-1-a endometrial epithelial cancinoma cell line				
With hCGβ added	479 ± 25 (100%)	558 ± 6.0 (116%)	586 ± 34 (122%)	$P = 0.011$
With hCGβ-H added	479 ± 25 (100%)	554 ± 49 (116%)	563 ± 25 (118%)	$P = 0.015$

131%, 155% and 122%, respectively. While hCG-H promoted these same cells to grow to 142%, 114%, 148% and 129%. No significant difference is noted in the hCGβ and hCG-H actions by *t*-test ($P = 0.56$).

We know from this study and from previous studies that hCG-H promotes choriocarcinoma (Cole et al., 2006a; Guibourdenche et al., 2010), and that hCGβ or hCGβ-H promotes all other malignancies (Butler and Iles, 2010; Iles, 2007; Li et al., 2008). These are multiple variants and dissociation products of hCG promoting cancer growth. We asked whether the root molecule hCG has any function (Table 4). All 6 cell lines, the 2 choriocarcinoma cell lines and the 4 other cell lines, were incubated with 0.27 nmol/L pure hCG. As found, hCG had no significant or measurable effect (100%, 97%, 95%, 102%, 98% and 103%, respectively) on any cell line. By *t*-test no significant action detected ($P = 0.51$).

B152 is an antibody to neutralize hCG-H/hCGβ-H actions. Cells were incubated with 0.5–10 μg/ml B152. As shown, B152, 10 μg/ml, reduced cell growth significantly (Table 5). Decreasing growth to 66%, 67%, 65%, 69%, 78% and 65% of 0 μg/ml control flasks, or reducing growth significantly by *t*-test, $P = 0.0053$, $P = 0.011$, $P < 0.0005$, $P = 0.014$, $P < 0.005$ and $P = 0.0010$, respectively.

4. Discussion

It is concluded that the three hCG-related cancer promoters, hCG-H, hCGβ and hCGβ-H, are interchangeable, all promoting cell

Please cite this article in press as: Cole, L.A., Butler, S. Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ: Interchangeable cancer promoters. Molecular and Cellular Endocrinology (2011), doi:10.1016/j.mce.2011.10.029

Table 3
The action of hCG-H and hCGβ on growth of Jar, JEG-3, SCaBER, T24, KLE and Hec-1-a cancer cells. Cells were cultured in T-25 flasks to 70% flask confluency (24 flasks per cell line, 6 conditions in quadruplicate) and then cultured 24 h with the addition of hCG, 0, 0.027 and 0.27 nmol/L, hCG-H, 0, 0.027 and 0.27 nmol/L and hCGβ, 0, 0.027 and 0.27 nmol/L (4 flasks each). All flasks were then cell counted in quadruplicate. Results are mean counts (× 1000) ± standard deviation.

Cell line and additives	Cell count after 24 h culture (× 1000)			*t*-Test, 0.27 nmol/L vs. 0 nmol/L
	0 nmol/L	0.027 nmol/L	0.27 nmol/L	
1. JArchoriocarcinoma cell line				
With hCG-H added	323 ± 3.5 (100%)	414 ± 54 (106%)	455 ± 6.4 (127%)	*P* = 0.020
With hCGβ added	323 ± 3.5 (100%)	370 ± 15 (115%)	478 ± 12 (148%)	*P* = <0.00005
2. JEG-3 choriocarcinoma cell line				
With hCG-H added	652 ± 57 (100%)	705 ± 13 (108%)	737 ± 25 (113%)	*P* = 0.077
With hCGβ added	652 ± 57 (100%)	738 ± 31 (113%)	755 ± 15 (116%)	*P* = 0.039
3. SCaBER bladder epithelial carcinoma cell line				
With hCG-H added	366 ± 18 (100%)	476 ± 79 (130%)	520 ± 38 (142%)	*P* = 0.0032
With hCGβ added	366 ± 18 (100%)	441 ± 41 (120%)	488 ± 33 (133%)	*P* = 0.0049
4. KLE endometrial adenocarcinoma cell line				
With hCG-H added	417 ± 7.1 (100%)	436 ± 32 (105%)	476 ± 29 (114%)	*P* = 0.027
With hCGβ added	417 ± 7.1 (100%)	510 ± 7.2 (122%)	547 ± 14 (131%)	P= 0.0001
5. T24 bladder epithelial carcinoma cell line				
With hCG-H added	708 ± 12 (100%)	872 ± 102 (123%)	1045 ± 7.1 (148%)	*P* = <0.00005
With hCGβ added	708 ± 12 (100%)	842 ± 48 (119%)	1095 ± 34 (155%)	*P* = <0.00005
6. Hec-1a endometrial epithelial cancinoma cell line				
With hCG-H added	479 ± 25 (100%)	531 ± 26 (109%)	619 ± 23 (129%)	*P* = 0.0020
With hCGβ added	479 ± 25 (100%)	558 ± 6.0 (116%)	586 ± 34 (122%)	P=0.011

Table 4
The action of hCG on growth of Jar, JEG-3, SCaBER, T24, KLE and Hec-1-a cancer cells. Cells were cultured in T-25 flasks to 70% flask confluency (12 flasks per cell line, 3 conditions in quadruplicate) and then cultured 24 h with the addition of hCG, 0, 0.027 and 0.27 nmol/L(4 flasks each). All flasks were then cell counted in quadruplicate. Results are mean counts (× 1000) ± standard deviation.

Cell line and additives	Cell count after 24 h culture (× 1000)			*t*-Test, 0.27 nmol/L vs. 0 nmol/L
	0 nmol/L	0.027 nmol/L	0.27 nmol/L	
1. JArchoriocarcinoma cell line				
With hCG added	323 ± 3.5 (100%)	289 ± 40 (89%)	324 ± 50 (100%)	*P* = 0.98
2. JEG-3 choriocarcinoma cell line				
With hCG added	652 ± 57 (100%)	681 ± 43 (104%)	631 ± 31 (97%)	*P* = 0.60
3. SCaBER bladder epithelial carcinoma cell line				
With hCG added	406 ± 12 (100%)	414 ± 51 (99%)	400 ± 40 (95%)	*P* = 0.78
4. KLE endometrial adenocarcinoma cell line				
With hCG added	417 ± 7.1 (100%)	410 ± 7.5 (98%)	425 ± 2.1 (102%)	*P* = 0.13
5. T24 bladder epithelial carcinoma cell line				
With hCG added	1077 ± 25 1818 (100%)	1060 ± 64 (98%)	1054 ± 35 (98%)	*P* = 0.96
6. Hec-1a endometrial epithelial cancinoma cell line				
With hCG added	479 ± 25 (100%)	461 ± 60 (96%)	485 ± 60 (103%)	*P* = 0.88

growth in choriocarcinoma and other cancer cells. As shown, hCGβ antagonizes cancer cells leading to a blockage of apoptosis (Butler et al., 2000; Butler and Iles, 2003; Carter et al., 2006; Cosgrove et al., 1989; Iles, 2007) through known pathways which may involvecystine knot growth factors and their receptors. An abundance of research indicates that hCG-H may also bind to a similar receptor (Fisher et al., 1989; Hwang et al., 2001; Kamijo et al., 1998; Kingsley-Kallesen et al., 1997; Knittel et al., 1999; Liu et al., 2005; Murphy et al., 1987; Pampferf 2000; Pringle and Roberts, 2007; Qureshi et al., 2005; Schuster and Krieglstein, 2002; Shooner et al., 2005; Staun-Ram and Shaleu, 2005; Stetler-Stevenson et al., 1990). The findings reported here clearly indicate that all

Please cite this article in press as: Cole, L.A., Butler, S. Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ: Interchangeable cancer promoters. Molecular and Cellular Endocrinology (2011), doi:10.1016/j.mce.2011.10.029

Table 5
The action of B152 anti-hCG-H/hCGβ-H on growth of Jar, JEG-3, SCaBER, T24, KLE and Hec-1-a cancer cells. Cells were cultured in T-25 flasks to 70% flask confluency (12 flasks per cell line, 3 conditions in quadruplicate) and then cultured 24 h with the addition of antibody, 0, 0.5, 1, 2 and 10 μg/ml (4 flasks each). All flasks were then cell counted in quadruplicate. Results are mean counts (× 1000) ± standard deviation.

Cell line and additives	Cell count after 24 h culture (× 1000)					*t*-Test, 10 μg/ml vs. 0 μg/ml
	0 μg/ml	0.5 μg/ml	1.0 μg/ml	2.0 μg/ml	10 μg/ml	
1. JArchoriocarcinoma cell line						
With antibody B152 added	465 ± 23 (100%)	407 ± 6.4 (88%)	365 ± 18 (78%)	324 ± 3.5 3.5 (69%)	310 ± 43 (66%)	*P* = 0.0053
2. JEG-3 choriocarcinoma cell line						
With antibody B152 added added	547 ± 62 (100%)	513 ± 43 (94%)	483 ± 47 (88%)	421 ± 18 (77%)	368 ± 33 (67%)	*P* = 0.011
3. SCaBER bladder epithelial carcinoma cell line						
With antibody B152 added	492 ± 10 (100%)	468 ± 20 (95%)	416 ± 19 (85%)	360 ± 37 (73%)	320 ± 19 (65%)	*P* = <0.0005
4. KLE endometrial adenocarcinoma cell line						
With antibody B152 added	460 ± 54 (100%)	412 ± 13 (90%)	398 ± 26 (87%)	374 ± 18 (81%)	316 ± 20 (69%)	*P* = 0.014
5. T24 bladder epithelial carcinoma cell line						
With antibody B152 added	1353 ± 28 (100%)	1227 ± 45 (91%)	1168 ± 16 (86%)	1128 ± 48 (83%)	1059 ± 35 (78%)	*P* = <0.0005
6. Hec-1a endometrial epithelial cancinoma cell line						
With antibody B152 added	547 ± 2.0 (100%)	494 ± 26 (90%)	428 ± 54 (78%)	406 ± 17 (74%)	357 ± 38 (65%)	*P* = 0.0010

promoters are seemingly interchangeable, working on the same receptor or at least through similar pathways. Research shows that hCG β-subunit has common evolutionary origins with TGFβ (Laub and Jennissen, 2003; Lehnert and Ankhurst, 1988), and that hCG crystal structure contains a cystine knot structure with 4 peptides common to hCG β-subunit TGFβ, PDFG and NGF (Lapthorn et al., 1994).

It is true that regular hCG contains evolutionary sequences and structures common to TGFβ, PDFG and NGF. We question why it is that regular hCG, as shown, has no function in any of the autocrine actions described here. We believe that with complex folding these growth factor sites become sterically hidden in regular hCG (Lapthorn et al., 1994). The molecules produced in cancer may not share the same conformations of regular hCG and that in processing as lone hCGβ subunits and with additional glycosylation they may expose receptor binding regions. Just as both hCG-H or hCGβ-H can stimulate either choriocarcinoma or other cancers, antibody B152 can block them. This antibody binds hCG-H and hCGβ-H, significantly limiting growth of the 2 choriocarcinoma and the 4 other cell lines.

A vaccine has been generated to hCGβ. The antibody generated in response to this vaccine should bind equally hCG-H, hCGβ and hCGβ-H. This vaccine is used for advanced malignancies. Clinical trials show that this vaccine can have a significant effect on cancer growth leading to doubling of cancer longevity (Delves et al., 2007; Iversen et al., 2003; Morse et al., 2011; Moulton et al., 2002) and there is clearly great potential for anti hCGβ vaccines (Butler and Iles, 2010). It appears from this study that hCGβ and hCGβ-H may be a principal non-placental non-germ cell cancer promoters in advanced malignancies.

How do the two types of malignancy, choriocarcinoma and germ cell malignancies which produce hCG-H, and other malignancies which produce hCGβ and hCGβ-Hdiffer? Choriocarcinoma and germ cell malignancies are primarily cytotrophoblastic in histology, The cells are eutopic cells or the root cell cells for producing hCG-H (Cole et al., 2006a,b). As such, these cancers are driven by these cells and hCG-H in both early and advanced stages.

In contrast, all other malignancies, which produce hCGβ and hCGβ-H, are produced by cells other than trophoblast cells. These cells are ectopic, in that they do not naturally secrete an hCG form. It is only when the malignancy becomes advanced that the necessary cell differentiation occurs and root trophoblast genes, with the *CGβ* gene cluster, are again expressed ectopically (Bellet et al., 1997; Butler et al., 2000; Butler and Iles, 2003; Carter et al., 2006; Cosgrove et al., 1989; Iles, 2007; Butler and Iles, 2010). These cells may not be able to secrete hCG subunit dimer and expression of just CGβ genes independent of CGα expression leads to sufficient synthesis of hCGβ and hCGβ-H. As indicated here, hCGβ and hCGβ-H cause similar antagonizationto hCG-H and it likely to be via the same pathway. It is inferred, that multiple as yet undefined promoters and pathways control these cancers in early stages. When a cancer becomes advanced, such that the cells have reached a state of differentiation whereby they can express the ectopic hCG β-subunit genes, this becomes a key cancer promotion pathway.

Thus we have two kinds of cancer: that which is continuously controlled by hCG-H, such as choriocarcinoma and germ cell malignancies and that which is only controlled by hCGβ and hCGβ-H in advanced stage, epithelial malignancies. These two models may be defined as EutopichCG producing cancers and ectopic hCG producing cancers and while vaccines and antibodies to hCGβ may bring about potentialtherapy for ectopic malignancies, they may also be able to play a significant role in the treatment ofeutopic malignancies. We know that a company, Omnimmune Corp., holds the licence on antibody B152. It is currently trying to develop a humanized B152. Such an antibody could ultimately be used as a possible cure for eutopic placental and germ cell malignancies, and as a major treatment for advanced ectopic or other malignancies.

For 14 years now separate research and discoveries have been ongoing throughout the world. Some scientists discovered that placental cytotrophoblast cells produce hCG-H. This was shown to promote growth of the cells that produce it, promote pregnancy implantation, and choriocarcinoma and germ cell cancer growth and invasion (Cole et al., 2006a,b; Guibourdenche et al., 2010; Handschuh et al., 2007a,b). Other scientists discovered that hCGβ and hCGβ-H are produced by non-trophoblastic neoplasms, and that they promote growth and malignancy in these cancer cells (Bellet et al., 1997; Butler and Iles, 2003, 2010; Butler et al., 2000; Carter et al., 2006; Cosgrove et al., 1989; Iles, 2007; Li et al., 2008). Here we bridge the two groups by showing for the first time that all 3 promoter molecules, hCG-H, hCGβ and hCGβ-

Please cite this article in press as: Cole, L.A., Butler, S. Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ: Interchangeable cancer promoters. Molecular and Cellular Endocrinology (2011), doi:10.1016/j.mce.2011.10.029

H can do the same things in all types of cell lines, and so all discoveries are seemingly related.

Here we infer the mode of action of hCG-H, hCGβ, and hCGβ-H involves antagonism of a TGFβ receptor. This needs to be confirmed by showing that all 3 molecules do bind the TGFβ receptor. This will require receptor isolation studies. There are 3 established TGFβ receptors, TGFβRI, -RII and-RIII. Published data suggest the involvement of TGFβ RII or TGFβ RIII receptors in hCG-H, hCGβ, and hCGβ-H actions (Butler et al., 2000; Khoo et al., 1998; Kingsley-Kallesen et al., 1997; Qureshi et al., 2005; Schuster and Krieglstein, 2002; Shooner et al., 2005). This needs to be confirmed and the correct TGFβ receptor identified.

5. Uncited reference

Mizuochi et al. (1985).

References

Acevedo, H.F., Hartstock, R.J., 1996. Metastatic phenotype correlates with high expression of membrane-associated complete β-human chorionic gonadotropin in vivo. Cancer 78, 2388–2399.

Alfthan, H., Haglund, C., Robets, P., Stenman, U.H., 1992. Evaluation of free β-subunit of human choriogonadotropin and core β-fragment of human choriogonadotropin in the serum and urine of patients with malignant pancreatic and biliary disease. Cancer Res. 53, 4628–4633.

Bellet, D., Lazar, V., Bleche, I., Paradis, V., Giovangrandi, Y., Paterliru, P., 1997. Malignant transformation of nontrophoblastic cells in association with the expression of chorionic gonadotropin β genes normally transcribed in trophoblastic cells. Cancer Res. 57, 516–523.

Birken, S., Krichevsky, A., O'Connor, J., Schlatterer, J.A., Kardana, A., Cole, L.A., Canfield, R., 1999. Development and characterization of antibodies to a nicked and hyperglycosylated form of hCG from a choriocarcinoma patient: generation of antibodies that differentiate between pregnancy hCG and choriocarcinomahCG. Endocrine 10, 137–144.

Butler, S.A., Ikram, M.S., Mathieu, S., Iles, R.K., 2000. 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 82, 1553–1556.

Butler, S.A., Staite, E.M., Iles, R.K., 2003. Reduction of Bladder cancer cell growth in response to hCGβ vaccinated mouse serum. Oncol. Res. Anti Cancer Drug Des. 14, 93–100.

Butler, S.A., Iles, R.K., 2003. Ectopic human chorionic gonadotrophin β secretion by epithelial tumors and human chorionic gonadotrophin β-induced apoptosis in Karposi's sarcoma, is there a connection? Clin. Cancer Res. 9, 4666–46732.

Butler, S.A., Iles, R.K., 2010. Biological function of the free ÃŸ-subunit: expression and treatment target in cancer. In: Cole, L.A. (Ed.), Human Chorionic Gonadotropin (hCG). Elsevier Inc, Burlington MA, pp. 153–172.

Carter, W.B., Sekharem, M., Coppola, D., 2006. Human chorionic gonadotropin induces apoptosis in breast cancer. Breast Cancer Res. Treat. 100, S243–S244.

Cole, L.A., Hussa, R.O., 1981. Use of glycosidase digested human chorionic gonadotropin β-subunit to explain the partial binding of glycoprotein hormones to Con A. Endocrinology 109, 2276–2279.

Cole, L.A., 1987. The O-linked oligosaccharides are strikingly different on pregnancy and choriocarcinomahCG. J. Clin. Endocrinol. Metab. 65, 811–813.

Cole, L.A., Birken, S., Sutphen, S., Hussa, R.O., Pattillo, R.A., 1982. Absence of the COOH-terminal peptide on ectopic human chorionic gonadotropin β-subunit (hCG-β). Endocrinology 110, 2198–2200.

Cole, L.A., 1994. Urinary Beta Core Fragment (β-core, UGP or UGF). Tumor Marker Upd. 6, 69–75.

Cole, L.A., Sutton, J.M., Higgins, T.N., Cembrowski, G.S., 2004. Between-method variation in hCG test results. Clin. Chem. 50, 874–882.

Cole, L.A., Dai, D., Butler, S.A., Leslie, K.K., Kohorn, E.I., 2006a. Gestational trophoblastic diseases: 1. Pathophysiology of hCG-H-regulated neoplasia. Gynecol. Oncol. 102, 144–149.

Cole, L.A., Khanlian, S.A., Riley, J.M., Butler, S.A., 2006b. hCG-H (hCG-H) in gestational implantation, and in choriocarcinoma and testicular germ cell malignancy tumorigenesis. J. Reprod. Med. 51, 919–929.

Cole, L.A., 2007. hCG-H. Placenta 28, 977–986.

Cole, L.A., Khanlian, S.A., Muller, C.Y., 2008. Blood test for placental site trophoblastic tumor (PSTT) and for non-trophoblasticmalignancy for evaluating patients with low positive hCG results. J. Reprod. Med. 53, 457–464.

Cole, L.A., Laidler, L., Muller, C., 2010. USA hCG reference service, 10-year report. Clin. Biochem. 43, 1013–1022.

Cosgrove, D.E., Campain, J.A., Cox, G.S., 1989. Chorionic gonadotropin synthesis by human tumor cell lines: examination of subunit accumulation steady-state levels of mRNA and gene structure. Biochem. Biophys. Acta 1007, 44–54.

Delves, P.J., Iles, R.K., Roitt, I.M., Lund, T., 2007. Designing a new generation of anti-hCG vaccines for cancer therapy. Mol. Cell. Endocrinol., 260–276, 281.

Elliott, M.M., Kardana, A., Lustbader, J.W., Cole, L.A., 1997. Carbohydrate and Peptide structure of the α- and β-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. Endocrine 7, 15–32.

Fisher, S.J., Tian-yi, C., Li, Z., Hartman, L., Grahl, K., Zhang, G.-Y., Tarpey, J., Damsky, C.H., 1989. Adhesive and degradative properties of human placental cytotrophoblast cell in vitro. J. Cell Biol. 109, 891–902.

Guibourdenche, J., Handschuh, K., Tsatsaris, V., Gerbaud, M.C., Legul, F., Muller, D., Evain-Brion, D., Fournier, T., 2010. hCG-His a marker of earlyhumantrophoblast invasion. J. Clin. Endocrinol. Metab. 95, E240–E244.

Handschuh, K., Guibourdenche, J., Tsatsaris, V., Guesnon, M., Laurendeau, I., Evain-Brion, D., Fournier, T., 2007a. Humanchorionicgonadotropin expression in humantrophoblastsfromearly placenta, comparative studybetweenvillous and extravilloustrophoblasticcells. Placenta 28, 175–184.

Halila, H., Alfthan, H., Stenman, U.H., 1989. Clinical use of gynaecological tumor markers. Annal. Chir. Gynaecol. 78, 65–70.

Handschuh, K., Guibourdenche, J., Tsatsari, V., Guesnon, M., Laurendeau, I., Evain-Brion, D., Fournier, T., 2007b. Humanchorionic gonadotropin produced by the invasive trophoblast but not the villoustrophoblast promotes cell invasion and is down-regulated by peroxisomeproliferator-activatedreceptor-a. Endocrinology 148, 5011–5019.

Hussa, R.O., Fein, H.G., Pattillo, R.A., Nagelberg, S.B., Rosen, S.W., Weintraub, B.D., Perini, F., Ruddon, R.W., Cole, L.A., 1986. A distinctive form of human chorionic gonadotropin beta-subunit-like material produced by cervical carcinoma cells. Cancer Res. 46, 1948–1954.

Hwang, J.H., Koh, S.H., Han, D.I., Chung, S.R., Park, M.I., Hwang, Y.Y., Jang, S.J., 2001. Expression of transforming growth factor-1, 2 in the decidua of the early pregnancy: Comparison of decidua basalis and decidua parietalis. Korean J. Obstet. Gynecol. 44, 1145–1149.

Iles, R.K., 2007. Ectopic hCGβ expression by epithelial cancer: Malignant behaviormetastasis and inhibition of tumor cell apoptosis. Mol. Cell. Endocrinol. 260, 264–270.

Iversen, P.L., Mourich, D.V., Moulton, H.M., 2003. Monoclonal antibodies to two epitopes of β-human chorionic gonadotropin for the treatment of cancer. Cur. Opin. Mol. Thera. 5, 156–160.

Kamijo, T., Rajabi, M.R., Mizunuma, H., Ibuki, Y., 1998. Biochemical evidence for autocrine/paracrine regulation of apoptosis in cultured uterine epithelial cells during mouse embryo implantation in vitro. Mol. Hum. Reprod. 4, 990–998.

Khoo, N.K., Bechberger, J.F., Shepherd, T., Bond, S.L., McCrae, K.R., Hamilton, G.S., Lala, P.K., 1998. SV40 Tag transformation of the normal invasive trophoblast results in a premalignant phenotype I Mechanisms responsible for hyperinvasiveness and resistance to anti-invasive action of TGFβ. Int. J. Cancer 77, 429–439.

Kingsley-Kallesen, M., Johnson, L., Scholtz, B., Kelly, D., Rizzino, A., 1997. Transcriptional regulation of the TGF-beta 2 gene in choriocarcinoma cells and breast carcinoma cells: differential utilization of Cis-regulatory elements. In Vitro Cell Dev. Bio. Anim. 33, 294–301.

Knittel, T., Mehde, M., Kobold, D., Saile, B., Dinter, C., Ramadori, G., 1999. Expression patterns of matrix metalloproteinases and their inhibitors in parenchymal and non-parenchymal cells of rat liver regulation by TNF-alpha and TGF-beta1. J. Hepatol. 30, 48–60.

Lapthorn, A.J., Harris, D.C., Littlejohn, A., Lustbader, J.W., Canfield, R.E., Machin, K.J., 1994. Crystal structure of hCG. Nature 369, 455–461.

Laub, M., Jennissen, H.P., 2003. Identification of the anthelix motif in the TGF-β superfamily by molecular 3D-Rapid Prototyping. Materialwissenschaft und Werkstofftechnik 34, 1113–1119.

Lehnert, S.A., Ankhurst, R.A., 1988. Embryonic expression pattern of TGF beta type-1 RNA suggests both paracrine and autocrine mechanisms of action. Development 104, 263–273.

Li, D., Wen, X., Ghali, L., Al-Shalabi, F.M., Docherty, S.M., Purkis, P., Iles, R.K., 2008. hCGβ expression by cervical squamous carcinoma – in vivo histological association with tumor invasion and apoptosis. Histopathology 53, 147–155.

Liu, Y.-X., Gao, F., Wei, P., Chen, X.-L., Gao, H.-J., Zou, R.-Z., Siao, L.-J., Xu, F.-H., Feng, Q., Liu, K., Hu, Z.-Y., 2005. Involvement of molecules related to angiogenesis, proteolysis and apoptosis in implantation in rhesus monkey and mouse. Contraception 71, 249–262.

Martell, R.E., Ruddon, R.W., 1990. Patterns of human chorionic gonadotropin expression in untreated and 8-bromoadenosine-treated JAR choriocarcinoma cells. Endocrinology 126, 2757–2764.

Mizuochi, T., Nishimura, R., Taniguchi, T., Utsunomiya, T., Mochizuki, M., Derappe, C., Kobata, A., 1985. Comparison of carbohydrate structure between human chorionic gonadotropin present in urine of patients with trophoblastic diseases and healthy individuals. Jpn. J. Cancer Res. 76, 752–759.

Morse, M.A., Chapman, R., Powderly, J., Blackwell, K., Keler, T., Green, J., Riggs, R., He, L.Z., Ramakrishna, V., Vitale, L., Zhao, B., Butler, S.A., Hobeika, A., Osada, T., Davis, T., Clay, T., Lyerly, H.K., 2011. Phase I study utilizing a novel antigen-presenting cell-targeted vaccine with toll-like receptor stimulation to induce immunity to self-antigens in cancer patients. Clin. Cancer Res. 17, 4844–4853.

Moulton, H.M., Yoshihara, P.H., Mason, D.H., Iversen, P.L., Triozzi, P.L., 2002. Active specific immunotherapy with β-human chorionic gonadotropin peptide vaccine in patients with metastatic colorectal cancer: antibody response is associated with improved survival. Clin. Cancer Res. 8, 2044–2051.

Murphy, G., Reynolds, J.J., Whitham, S.E., Docherty, A.J., Angel, P., Heath, J.K., 1987. Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. Eur. Mol. Biol. Org. J. 6, 1899–1904.

Pampferf, S., 2000. Apoptosis in rodent peri-implantation embryos, differential susceptibility of inner cell mass and trophectoderm cell lineages – a review. Placenta 21, S3–S10.

Please cite this article in press as: Cole, L.A., Butler, S. Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ: Interchangeable cancer promoters. Molecular and Cellular Endocrinology (2011), doi:10.1016/j.mce.2011.10.029

Pringle, K., Roberts, C., 2007. New light on early post-implantation pregnancy in the mouse: roles for insulin-like growth factor-II (IGF-II)? Placenta 28, 286–297.
Puisieux, A., Bellet, D., Troalen, G., Razafindratsita, A., Lhomme, C., Bohoun, C., Bidart, J-M., 1990. Occurrence of fragmentation of free and combined forms of the β-subunit of human chorionic gonadotropin. Endocrinology 126, 687–694.
Qureshi, H.Y., Sylvester, J., El Mabrouk, M., Zafarullah, M., 2005. TGF-beta-induced expression of tissue inhibitor of metalloproteinases-3 gene in chondrocytes is mediated by extracellular signal-regulated kinase pathway and Sp1 transcription factor. J. Cell Physiol. 203, 345–352.
Regelson, W., 1995. Have we found the "definitive cancer biomarker"? The diagnostic and therapeutic implications of human chorionic gonadotropin-beta statement as a key to malignancy. Cancer 76, 1299–1301.
Ruddon, R.W., Norton, S.E., 1993. Use of biological markers in the diagnosis of cancers of unknown primary tumor. Semin. Oncol. 20, 251–260.
Schuster, N., Krieglstein, K., 2002. Mechanisms of TGF-β-mediated apoptosis. Cell Tissue Res. 307, 1–14.
Shooner, C., Caron, P.C., Fréchette-Frigon, G., Leblanc, V., Déry, M.-C., Asselin, E., 2005. TGF-β expression during rat pregnancy and activity on decidual cell survival. Reprod. Biol. Endocrinol. 3, 20.
Staun-Ram, E., Shaleu, E., 2005. Human trophoblast function during the implantation process. Reprod. Biol. Endocrinol. 3, 56–68.
Stetler-Stevenson, W.G., Brown, P.D., Onisto, M., Levy, A.T., Liotta, L.A., 1990. Tissue inhibitor of metalloproteinases-2 (TIMP-2) mRNA expression in tumor cell lines and human tumor tissues. J. Biol. Chem. 265, 13933–13938.
Valmu, L., Alfthan, H., Hotakainen, K., Birken, S., Stenman, U.H., 2006. Site-specific glycan analysis of human chorionic gonadotropin {beta}-subunit from malignancies and pregnancy by liquid chromatography – electrospray mass spectrometry. Glycobiology 16, 1207–1218.

Please cite this article in press as: Cole, L.A., Butler, S. Hyperglycosylated hCG, hCGβ and Hyperglycosylated hCGβ: Interchangeable cancer promoters. Molecular and Cellular Endocrinology (2011), doi:10.1016/j.mce.2011.10.029