HAL
open science

Does hCG or hCG play a role in cancer cell biology?

R.K Iles, P.J. Delves, S.A. Butler

To cite this version:

R.K Iles, P.J. Delves, S.A. Butler. Does hCG or hCG play a role in cancer cell biology?. Molecular and Cellular Endocrinology, 2010, 329 (1-2), pp.62. 10.1016/j.mce.2010.07.014 . hal-00627142

HAL Id: hal-00627142

https://hal.science/hal-00627142

Submitted on 28 Sep 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Does hCG or hCG play a role in cancer cell biology?

Authors: R.K Iles, P.J. Delves, S.A. Butler

PII: S0303-7207(10)00375-8
DOI: doi:10.1016/j.mce.2010.07.014
Reference: MCE 7601

To appear in: *Molecular and Cellular Endocrinology*

Received date: 7-2-2010
Revised date: 22-6-2010
Accepted date: 13-7-2010

Please cite this article as: Iles, R.K., Delves, P.J., Butler, S.A., Does hCG or hCG play a role in cancer cell biology?, *Molecular and Cellular Endocrinology* (2010), doi:10.1016/j.mce.2010.07.014

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

*Manuscript
Click here to view linked References
ACCEPTED MANUSCRIPT

Does hCG or hCGβ play a role in cancer cell biology?

R.K Iles[†], P. J. Delves[#] & S.A. Butler [†]

[†]Centre for Investigative and Diagnostic Oncology, Middlesex University, UK and
[#]Division of Infection and Immunity, University College London, London, UK

Keywords: hCG, hCGß, differentiation, oncogenesis, apoptosis, cancer

[*] Correspondence address:
Professor Ray Iles,
Middlesex University
The Burroughs
Hendon
London NW4 4BT
Te: +44 (0)20 8411 6577/5440
email: r.iles@mdx.ac.uk

Accepted Manuscript

ACCEPTED MANUSCRIPT

Summary

The role that hCG might play in the oncogenic process in cancer is certainly complex. We know that the expression of hCG and its beta subunit is a widespread phenomenon which has been described in many cancer subtypes. However, hCG's involvement in breast cancer has been antithetical: the detection of ectopically expressed hCG(β) by breast tumors has been employed as a biomarker of malignancy, and hCG has been proposed as a ligand vehicle for toxic drugs, with the aim of targeting the LH/hCG receptor which is reported to be expressed by malignant breast tissue. However, it has also been proposed that hCG is a protective agent against the development of breast cancer, leading some to advocate hCG administration to non-pregnant women as a prophylactic measure against cancer. Nevertheless, suggestions that hCG is involved in the angiogenesis, metastasis and immune escape that are central to cancer progression – are phenomena which clearly apply to breast cancer. Indeed, a tumor vaccine based upon hCG has very recently been shown to protect against mammary tumors in mice. We propose that this apparent paradox is resolved if the free **beta** subunit of hCG produced by tumors acts as an autocrine anti-apoptotic and angiogenic growth factor, whilst intact heterodimeric hCG, as in pregnancy, is part of developmental signaling that initiates tissue differentiation (including breast ductal tissue development), and hence reduces the population of stem-like cells which are susceptible to oncogenic factors.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Introduction

Ectopic human chorionic gonadotropin (hCG) expression by non-gestational tumors was noted as early as 1904 (DeJewitzi reported a case in which he concluded that a gonadotropin was produced by a bladder cancer – reviewed by Iles & Chard, 1991) and was reported by Ascheim and Zondek following the introduction of their seminal bioassay for hCG in 1927 (Ascheim & Zondek, 1927; Ascheim & Zondek, 1928; Zondek, 1930; Zondek, 1935). Up to the late 1970s, the incidence of ectopic hCG expression by common epithelial cancers could only be gleaned from a number of case reports. However, since the 1980s publications describing the ectopic expression of hCG have grown exponentially, and such findings are now generally reported in terms of the percentage of hCG positive patients with a given type of epithelial cancer (reviewed by Iles and Butler, 1998). A major reason for this improved appreciation of the frequent occurrence of ectopic hCG – and, in particular, hCGβ, expression - in many cancers was the introduction by Vaitukaitis *et al.* in 1972 of a radioimmunoassay which detects the free β-subunit of hCG, in addition to the intact hormone. Extensive immunochemical characterization has shown that, whilst intact hCG is abundantly produced by the placenta and by germ cell tumors, it is the free β subunit (independently of the common glycoprotein hormone α subunit [GPHα] that is predominantly produced by common epithelial tumors (Iles & Chard, 1989; Iles *et al.*, 1990a, Iles *et al.*, 1990b, Stenman et al 2004). Ectopic production of free hCGβ by bladder carcinoma is well established (reviewed by Iles & Butler, 1998, Hotakainen *et al* 2002b), and its expression has also been shown in a broad range of common epithelial cancers including breast, cervical, endometrial, prostate, lung, colon, oral/facial tissue and stomach (Table 1). The incidence is very variable but on

ACCEPTED MANUSCRIPT

average ~30% of epithelial cancer patients will have elevated serum levels, 37% elevated urinary levels and 48% of tumors will stain hCGβ positive. Intact hCG heterodimer may also be produced by various common epithelial tumors in an unpredictable fashion, most often by lung cancer and hepatoblastoma (Stenman *et al*., 2004). In bladder cancer, the expression of the hCG heterodimer is associated with carcinomatosis. However, the free β-subunit is the most abundant form of immunoreactive hCG expressed by non-germ cell tumors (Iles and Chard, 1991). An association between hCGβ expression, and anaplastic and ~~advanced~~ disease, has been noted in virtually all the published studies (Table 1) and many authors have commented on the aggressive nature of the hCGβ-positive tumors (Reviewed previously, Iles, 2007)

The biological action of hCGβ on epithelial tumors

The association of hCGβ expression by epithelial cancers with a tendency for the tumor to resist radiotherapy and develop metastases has, in the past, been largely dismissed as an epiphenomenon of no functional significance for oncogenesis and/or tumor spread. Thus hCG (and by association, hCGβ) expression has largely been regarded solely as a marker of the presence of pluripotent stem/germ cells. In 1996, it was demonstrated that bladder cancer cell numbers increased in a dose-dependent manner following incubation with hCGβ (Gillott *et al*., 1996). No effect could be seen following treatment with intact hCG, GPHα or hCGβ core fragment (hCGβcf, a naturally-occurring proteolytic cleavage fragment of hCGβ), but the growth stimulation was abolished by co-incubation with anti-hCGβ antiserum (Gillott *et al*.,1996). Furthermore, addition of these antibodies to the media used to culture bladder cancer cell lines inhibited the growth of the 5637, ScaBER and RT112

ACCEPTED MANUSCRIPT

cell lines that themselves produce free hCGβ, but did not affect the growth of the T24 bladder cell line that does not produce hCGβ (Gillott *et al.*, 1996). It was also noted that the cell lines least affected by the β-subunit were those that secreted the highest concentrations of hCGβ, suggestive of autocrine stimulation. The hCGβ/hLHβ gene cluster has been shown not to be amplified or rearranged in bladder tumor cells, indicating that ectopic hCGβ expression is more likely to be the result of altered gene regulation (Iles *et al.*, 1989). The inhibitory effects of anti-hCG antibodies on the growth of tumor cells has also been demonstrated *in vivo* in athymic (nude) mice with choriocarcinoma (Bagshawe *et al*., 1988). Furthermore, anti-sense oligonucleotides directed to the hCG β-chain have antiproliferative effects on prostate and cervical tumor cell lines *in vitro* (Devi *et al*., 2002; Jankowska et al., 2008).

Other studies showed that the increase in the cancer cell population in response to hCGβ was brought about by inhibition of apoptosis rather than by stimulation of cell replication (Butler *et al.*, 2000). Furthermore, given that the LH/hCG receptor is not expressed by these tissues, and that only intact hCG can stimulate the LH/hCG receptor, any activity observed must be mediated by an as yet unidentified pathway (Davies, 2001).

hCG is a member of the cystine knot growth factor/TGFβ superfamily

In 1994 the X-ray crystallographic structure of hCG was solved (Lapthorn *et al.*, 1994, Wu *et al*., 1994). The most striking feature was the arrangement of three disulfide bridges in the centre of each subunit. The positions of the three cystines are almost identical in both subunits, where two disulfides bridge the anti-parallel strands of the peptide chain

ACCEPTED MANUSCRIPT

forming a central loop, through which the third disulfide passes. This structure had been previously identified in a number of factors which are consequently designated the 'cystine knot growth factor' (CKGF) family. They include transforming growth factor β (TGFβ), platelet-derived growth factor B (PDGFB) and nerve growth factor (NGF). Since the identification of the structure of hCG in 1994, the family of growth factors has expanded, and now includes the vascular endothelial growth factors (VEGFs) and placental growth factor (PlGF) - despite sharing structural similarities the various members of the CKGF family carry out quite distinct functions (see figure 1). These additions to the CKGF family have further increased the intriguing possibilities for cross-talk between multiple growth regulatory systems, and ectopic expression of molecules containing a cystine knot may give rise to disruptions that favor cancer development. In fact, homology modeling using current data on the structure of hCG as obtained from crystal structure (Lapthorn *et al.,* 1994) reveals quite striking similarities to some CKGFs in particular VEGF and TGFβ (see figure 2).

Cross talk of hCGβ with CKGF receptors

The evolution of the hCG/LHβ gene cluster is fascinating, and the discovery that it is interspersed by another series of pseudogenes of the cystine knot growth factor family – neurotropin - further points to its origin from, and potential to interact with, other cystine knot growth and endocrine systems (Rull *et al.,* 2005) (see Figure 3)

Whilst the hCG heterodimer binds with high affinity (K_d 0.1–1.0 nM, Xie *et al*., 1990) to the CG/LH receptor, the free β subunit essentially does not stimulate the receptor. It has

ACCEPTED MANUSCRIPT

been demonstrated that the free subunits will also bind the LH receptor, albeit with a much lower affinity (Pierce & Parsons, 1981) and some studies have shown that upon receptor binding, the α subunit of hCG is internalized, and is associated with stimulation of adenylate cyclase activity. This implies that the binding model is not simple, and that hormone/receptor binding involves a topographical determinant extending across both ligand subunits (Ryan *et al.,* 1988). If intact hCG is required for LHR binding and stimulation, and LHR is not found in cell lines shown to respond to free hCGβ, then the biological activity described above for the free β subunit cannot be accounted for by signaling through this receptor. However, the structural homology with the cystine knot growth factors suggests that free hCGβ cross-interaction with other cystine knot growth factor receptors may occur.

One early feature of malignancy is neovascularisation, allowing the tumor to grow to a mass greater than 2mm. The process of vascularisation is regulated in part by cystine knot growth factors, in particular vascular endothelial growth factors (VEGFs). The development of blood vessels by the early and late placental tissue is distinct and involves VEGF receptor expression and stimulation (Demir *et al.,* 2004). Recent studies have suggested an interplay between placental VEGF and hCG in formation of placental vasularture (Herr *et al.,* 2007, Reisinger *et al.,* 2007). Indeed, hCG itself has been suggested to be a novel angiogenic factor (Zygmunt *et al.,* 2002) and we linked immunohistochemically detected hCGβ expression by cervical cancer with the extent of tumor vascularisation (Li *et al.,* 2008). In addition, Arrieta *et al* (2009) have recently linked serum hCG levels to neo-vascularisation of non-seminomatous testicular germ cell tumors. Therefore, Given the structural homology between hCG β and the cystine knot

ACCEPTED MANUSCRIPT

growth factors (lapthorn *et al.,* 1994), and its ability to dimerise (Butler *et al.,* 1999) cross talk with VEGF R is plausible.

TGFβ is encoded by a locus adjacent to that of the hCGβ-hLHβ gene cluster on chromosome 19 (Fujii, 1986), and high levels of TGFβ and its receptors are expressed by urothelial tumors, despite the growth inhibitory effects of this cytokine (Coombs *et al.,* 1993, Eder *et al.,* 1996, Eder *et al.,* 1997). This has long been a puzzle to cancer researchers; disruption of the TGFβ apoptosis signaling pathway has been considered an important feature of oncogenesis, i.e. escape from a fundamental mechanism of regulating the normal tissue epithelial cell population (Barcellos-Hoff & Ewan, 2000). We have previously shown that exogenous addition of hCGβ to bladder cancer cell cultures reverses the apoptosis-inducing effects of TGFβ (Butler *et al.,* 2000). Ectopic expression of free hCGβ may thus be acting as an autocrine/paracrine growth-stimulating factor by virtue of its ability to block TGFβ's action although we have never published receptor binding data. Its structural homology to TGFβ suggests that free hCGβ may bind to a component of the TGFβ receptor complex (for example, TGFRII) or affect other downstream apoptopic signals.

Therapeutic targeting and selection of hCGβ expressing tumors

The molecular pathway proposed above is a reversal of the Her2 - Herceptin® molecular mechanism, but the parallels are somewhat compelling. The step that propelled Her2 from being a marker of poor prognosis was that it acted as both the selection criteria for a

ACCEPTED MANUSCRIPT

therapy, and was itself the target of a novel humanized antibody treatment. To date, no infusion of antibody has been attempted to treat hCGβ-expressing tumors, but an anti-hCGβ carboxy terminal peptide (CTP) vaccine - a much greater step - has completed a phase II clinical trial in patients with colorectal cancer (Moulton *et al*., 2002). A high anti-hCG titre response to the vaccine was associated with improved survival. However, several studies have indicated that only 15-41% of patients with colorectal cancer have increased levels of circulating hCGβ (Stenman, Alfthan & Hotakainen, 2004; Moulton *et al.*, 2002), and the vaccine was administered to patients irrespective of whether they were 'hCG positive' or not. A vaccine which stimulates immune responses against hCGβ would be expected to benefit only those patients in whom hCGβ is aberrantly expressed, suggesting that the vaccine might have benefited a higher proportion of the trial group if a lack of circulating hCG had been applied as an exclusion criterion for entry into the trial. Indeed, the Her2 paradigm clearly showed that Herceptin would never have displayed the small but significant advantage in breast cancer patient survival if it had been given to all breast cancer patients irrespective of their Her2 expression status (Baselga, 2001).

Taking many of these lessons on board Celldex Therapeutics (Needham, MA, USA) have developed an anti-hCGβ cancer vaccine based on their patented technology for targeting antigen presenting cells (APC) - (Keler *et al.*, 2007). Termed CDX-1307, this vaccine completed Phase 1 development and is marketed as a treatment for colorectal, pancreatic, bladder, ovarian and breast cancers. CDX-1307 couples the entire beta chain of hCG to an APC specific adjuvant antibody and induces both humoral and cell mediated response to the complete molecule and not just the CTP. Phase 2 study in patients with invasive

ACCEPTED MANUSCRIPT

bladder cancer began in 2010 but inclusion is dependant on demonstrating hCGβ expression by the tumors.

An hCG-specific anti-tumor vaccine engineered to optimize the immune response

The hCG vaccine administered in the phase II clinical trial in patients with colorectal cancer (Moulton *et al*., 2002) consisted of the CTP of hCGβ conjugated to diphtheria toxoid. Although - unlike the rest of the hCG β-chain - the CTP has the advantage of lacking sequence homology with the other members of the glycoprotein hormone family, it is known that it gives rise to relatively low affinity antibodies (Talwar, 1999). Furthermore, it clearly will possess a restricted range of hCG-specific epitopes compared to the full length β-chain, which contains a total of five unique epitopes: the linear epitopes β8 and β9 located within the CTP and the discontinuous epitopes β1, β6 and β7 found in the rest of the hCG β-chain (Berger *et al*., 2002). These epitopes are not present on other members (LH, FSH and TSH) of the glycoprotein hormone family. The other epitopes on the hCG β-chain are designated β2, β3, β4 and β5 and are cross-reactive with similar epitopes on the LH β-chain. Using oligonucleotide-directed site-specific mutagenesis, we made a number of amino acid replacements in the hCG β-chain with the aim of eliminating the cross-reactive epitopes (Jackson *et al*., 1996; Delves *et al*., 2007). One of the mutants, with only a single amino acid replacement (a glutamic acid substituted for the arginine at position 68), dramatically refocuses the immune response away from the immunodominant cross-reactive epitope region towards to hCG-specific β1, β6 β7 and CTP epitopes, with very little cross-reactive antibody production in both

ACCEPTED MANUSCRIPT

rabbit and mouse immunogenicity studies (Porakishvili *et al.*, 2002; Chiesa *et al.*, 2001). Strikingly, the antibodies produced in response to this mutant (hCGβR68E) bind avidly to the CTP (Porakishvili *et al.*, 2002). This molecule is therefore a candidate for an improved hCG-specific cancer vaccine.

The paradox

Ectopic expression of hCGβ serves as a molecular biomarker identifying a subpopulation of patient tumors with poor prognosis for which molecular mechanisms have been identified *in vitro* and which provides the basis of a therapeutic target for antibody therapy; similar to the Her2 Paradigm. Although this paradigm may hold for epithelial tumors such as bladder, cervix and lung, it is not as clear-cut for breast cancers. However, within the current scientific literature controversy exists over the role of the pregnancy hormone hCG in the pathogenesis of breast cancer. Whilst some have demonstrated that ectopic expression of hCGβ by the breast tumor itself is a marker of poor prognosis, others have proposed hCG to be a protective agent against the subsequent development of breast cancer.

Ectopic hCGβ expression by breast cancer as a marker of poor prognosis

Studies of ectopic hCG expression by breast cancer have been intriguing (Tables 2 & 3). Most have concentrated on immunohistochemical and mRNA expression as the method of detecting ectopic expression of hCG, specifically the beta-subunit. The incidence, combining all studies of immunohistochemical detection of ectopic expression by breast

ACCEPTED MANUSCRIPT

tumors, is low - 24% (148/621) - compared to the general incidence of ectopic expression by epithelial cancer described in table 1. Two of the five studies have specifically stated IHC detection of hCGβ expression by breast tumors does not to correlate with patient prognosis, while one eluded to prognosis as expression correlated with a specific histological type of breast cancer (apocrine) (Chang *et al.*, 2005).

Messenger RNA studies concentrated on looking for shed blood-circulating tumor cells, and used detection of hCGβ mRNA as one of the markers. Combining these studies, 36-38% of patients had circulating breast cancer cells shed from the primary tumor detected as expressing hCGβ mRNA (Table3). However, Hotakanien et al (2002b and 2007) have also suggested that false positives may arise due to poor primer design and result from LHβ expression by normal blood cells.

hCG as an anti-breast cancer therapy

The case for hCG being an agent with which to prevent cancer is encapsulated in José and Irma Russos' 2004 book, the *Molecular Basis of Breast Cancer: Prevention and Treatment.* The incidence of breast cancer is still rising, is the most prevalent cancer in Europe and USA, and epidemiology is increasingly indicating that many of the key risk factors are related to life-styles associated with Western cultures; refined food diet, early menarche, obesity, and that women have their first full-term pregnancies when they are older. Breast cancer is not common in some Far Eastern countries, although women from those societies are at greater risk if they migrate to the West than remain in their native country. Identifying how these risk factors impact on breast development at a molecular

ACCEPTED MANUSCRIPT

level could enable preventative strategies to be established. Russo and Russo focused on the observation that early pregnancy protects against breast cancer. This is not surprising as the breast epithelia dramatically alters during pregnancy, from a stem cell rich and dormant layer to a highly differentiated multiple-structured tissue capable of synthesizing and releasing large quantities of milk in response to key specific stimuli.

They go on to propose that a treatment to artificially induce differentiation within mammary epithelial cells could represent a radical new approach to prevent much of the disease. The epidemiological data from which this hypothesis arises is supported strongly by experimental observations that a state of pregnancy - whether induced naturally, by estrogen/progesterone treatment, or by human chorionic gonadotropin - has a dramatic inhibitory effect on carcinogen-induced mammary cancer in rats. The authors argue a case for ectopic administration of hCG as a possible preventative strategy. However, although this might be a viable strategy for eliminating tumors in rats, it might be premature to consider it as preventative in humans. The contentious issue in extrapolating the result from experimental studies on rats to humans is that the rat does not possess an equivalent hCG gene and their conceptae do not produce any gonadotropic-like hormone.

It is likely that the cell-differentiating properties of sustained exposure to ovarian steroids, as occurs in pregnancy, are part of a complex and coordinated temporal staging of pregnancy-associated cytokine signaling that induces breast epithelial stem cell differentiation towards more terminally developed lactational epithelia. The further along this pathway that differentiation proceeds, the greater the reduction in potential to develop breast cancer. More recent studies suggest that breast tissue expresses LH/hCG receptors and that hCG has the direct affect of human breast epithelial differentiation (see

Accepted Manuscript

ACCEPTED MANUSCRIPT

table 2). Indeed, for pregnancy-associated holo-hCG to have this effect, the breast epithelial tissue must express the LH/hCG receptor at functionally responsive levels.

Despite some reservations, phase I and II trials have been conducted on human patients with breast cancer receiving recombinant hCG (rhCG), and in December 2008 a phase III trial of patients at high risk for breast cancer receiving 30 doses of hCG was underway in New York (Prevention of Breast Cancer, randomized, single blind clinical trial (USA) – NCT00808522). Initial results should be published in 2010.

hCG and breast cancer

Interestingly several studies have used hCG as targeting ligand to which a toxin is conjugated (Bodek *et al.*, 2003; Hansel *et al.*, 2007). This suggests that such tumors express/over express LH/hCG receptors to which they respond. These two opposing positions raise the issue of whether exposure to exogenous hCG in the stimulated cycle protocol leading up to IVF have increased or decreased the risk of breast cancer. Reviewing the literature in a meta analysis of 60,050 women undergoing IVF cycles, Salhab and colleagues found that there was no change in relative risk (rr=1.03) for the development of breast cancer (Salhab *et al*., 2005) . Transgenic mice over-expressing an engineered LH which contains an hCG-like carboxy terminal peptide do have an increased incidence of breast cancer, but this is probably secondary to gross hyper-stimulation of ovarian function leading to anovulation, infertility, increased steroid production and ovarian granulosa cell tumorigenesis (Mann, Keri and Nilson 2003). In a similar vein of investigation, Chen *et al.*, (2009) examined polymorphisms around CGB gene 5 of the CG/LHβ gene cluster, and human placental lactogen gene of the hPL-GH

ACCEPTED MANUSCRIPT

gene cluster, in an attempt to link either hormone to the molecular causation of the 4.5 fold protective effect of term pregnancy on the risk of developing breast cancer. As a large case control study, subjects and controls were selected from the New York Breast Cancer Family Register. None of the polymorphic markers for hPL were associated with an increased risk of developing breast cancer. However, the variant C allele of *CGB5* rs726002 SNP had an elevated breast cancer risk (OR = 2.09) only in women bearing children at a young age, <26 years. Among women with a later age at childbirth, breast cancer risk did not differ.

Conclusion

Given the differences in structure and receptor target of intact hCG and free hCGβ, it is entirely feasible that the free beta subunit of hCG is produced by tumors and acts as an autocrine (anti-apoptotic) growth factor, whilst in pregnancy, intact heterodimeric hCG is part of a developmental signal that, among various systems, initiates breast ductal tissue differentiation and hence reduces the population of stem-like cells which are susceptible to oncogenic factors. The challenge is to truly discriminate these two actions as representing two distinct as yet to be defined molecular pathways.

Acknowledgement – we grateful acknowledge the 3D modelling of hCGβ dimers by Saif-ur-Rehman and his bioinformatics comparisons with the cystine knot superfamily. Professor Ray Iles is supported by the Lee Smith Foundation and *Peter Luck-Hille Foundation*

References

Accepted Manuscript

ACCEPTED MANUSCRIPT

Antony P.A., Piccirillo C.A., Akpinarli A., Finkelstein S.E., Speiss P.J., Surman D.R., Palmer D.C., Chan C.C., Klebanoff C.A., Overwijk W.W., Rosenberg S.A., Restifo N.P. 2004; $CD8^+$ T cell immunity against a tumor/self-antigen is augmented by $CD4^+$ T helper cells and hindered by naturally occurring T regulatory cells. J. Immunol. 174: 2591-2601.

Agnantis NJ, Patra F, Khaldi L, Filis S. 1992; Immunohistochemical expression of subunit beta HCG in breast cancer. Eur J Gynaecol Oncol. 13:461-6.

Arrieta O, Michel Ortega RM, Angeles-Sánchez J, Villarreal-Garza C, Avilés-Salas A, Chanona-Vilchis JG, Aréchaga-Ocampo E, Luévano-González A, Jiménez MA, Aguilar JL. 2009; Serum human chorionic gonadotropin is associated with angiogenesis in germ cell testicular tumors. J Exp Clin Cancer Res. 28:120.

Ascheim S., Zondek B. 1927; Hypophysenworderlappen hormon und ovarialhormon im Harn von Schwangeren. Klin. Wochenschr. 6: 1322–1324.

Ascheim C., Zondek B. 1928; Die Schwangerschaftsdiagnose aus dem Harn durch Nachweis der Hypophysevordem Lappenhormon. Klin. Wochenschr. **7**: 1404-1411.

Bagshawe K.D., Springer C.J., Searle F., Antoniw P., Sharma S.K., Melton R.G., Sherwood R.F. 1988; A cytotoxic agent can be generated selectively at cancer sites. Br. J. Cancer. 58: 700-703.

Barcellos-Hoff M.H., Ewan K.B.R. 2000; Transforming growth factor β and breast cancer: Mammary gland development. Breast Cancer Res. 2: 92-99.

Baselga J. 2001; Clinical trials of Herceptin(trastuzumab). Eur. J. Cancer. 37, Suppl 1: S18-24.

Berger P., Sturgeon C., Bidart J.M., Paus E., Gerth R., Niang M., Bristow A., Birken S., Stenman U.H. 2002; The ISOBM TD-7 Workshop on hCG and related molecules. Towards user-oriented standardization of pregnancy and tumor diagnosis: assignment of epitopes to the three-dimensional structure of diagnostically and commercially relevant monoclonal antibodies directed against human chorionic gonadotropin and derivatives. Tumour Biol. 23: 1-38.

Berzal-Cantalejo F., Sabater Marco V., Alonso Hernandez S., Jimenez Pena R., Martorell Cebollada M.A. 2004; Syncytial giant cell component. Review of 55 renal cell carcinomas. Histol. Histopathol. 19: 113-118.

Bhalang K., Kafrawy A.H., Miles D.A. 1999; Immunohistochemical study of the expression of human chorionic gonadotropin-beta in oral squamous cell carcinomas. Cancer 85: 757-762.

ACCEPTED MANUSCRIPT

Bieche I., Lazar V., Nogues C., Poynard T., Giovangrandi Y., Bellet D., Lidereau R., Vidaud M. 1998; Prognostic value of chorionic gonadotropin beta gene transcripts in human breast carcinoma. Clin. Cancer Res. 4: 671-676.

Bodek G, Rahman NA, Zaleska M, Soliymani R, Lankinen H, Hansel W, Huhtaniemi I, Ziecik AJ. A novel approach of targeted ablation of mammary carcinoma cells through luteinizing hormone receptors using Hecate-CGbeta conjugate. Breast Cancer Res Treat. 2003 May;79(1):1-10.

Boucher L.D., Yoneda K. 1995; The expression of trophoblastic cell markers by lung carcinomas. Hum. Pathol. 26: 1201-1206.

Butler S.A, Laidler, P.. Porter J.R,. Kicman A.T, Chard T.,. Cowen D.A. Iles R.K. 1999; The β subunit of human chorionic gonadotrophin exists as a homodimer. J. Mol Endocrinol, 22: 185-192.

Butler S.A., Ikram M.S., Mathieu S., Iles R.K. 2000; The increase in bladder carcinoma cell population induced by the free β subunit of human chorionic gonadotrophin is a result of an anti-apoptosis effect and not cell proliferation. Br. J. Cancer 82: 1553-1556.

Carlson H.E., Kane P., Lei Z.M., Li X., Rao C.V. 2004; Presence of luteinizing hormone/human chorionic gonadotropin receptors in male breast tissues. J. Clin. Endocrinol. Metab. 89: 4119-4123.

Carter P.G., Iles R.K., Neven P., Ind T.E.J., Shepherd J.H., Chard T. 1995; Measurement of urinary β-core fragment of human chorionic gonadotrophin in women with vulvovaginal malignancy and its prognostic significance. Br. J. Cancer 71: 350-353.

Chang E., Lee E., Oh S.J., Kim J.S., Kang C. 2005; The immunoexpressions and prognostic significance of inhibin alpha and Beta human chorionic gonadotrophins (HCG) in breast carcinomas. Cancer Res. Treat. 37: 241-246.

Chen Y., Kibriya M.G., Jasmine F., Santella R. M., Senie R.T., Ahsan H. 2009; Do Placental Genes Affect Maternal Breast Cancer? Association between Offspring's CGB5 and CSH1 Gene Variants and Maternal Breast Cancer Risk. Cancer Res. In press Author manuscript; available in PMC 2009 December 1.

Chiesa M.D., Martensen P.M., Simmons C., Porakishvili N., Justesen J., Dougan G., Roitt I.M., Delves PJ, Lund T. 2001; Refocusing of B-cell responses following a single amino acid substitution in an antigen. Immunology 103: 172-178.

Chopineau M, Stewart F, Allen WR. 1995; Cloning and analysis of the cDNA encoding the horse and donkey luteinizing hormone beta-subunits. Gene. 160: 253-6.

ACCEPTED MANUSCRIPT

Coombs L.M., Pigott D.A., Eydmann M.E, Proctor A.J., Knowles M.A. 1993; Reduced expression of TGFβ is associated with advanced disease in transitional cell carcinoma. Br. J. Cancer 67: 578-584.

Crawford R.A., Iles R.K., Carter P.G., Caldwell C.J., Shepherd J.H., Chard T. 1998; The prognostic significance of human chorionic gonadotrophin and its metabolites in women with cervical carcinoma. J. Clin. Pathol. 51: 685-688.

Daja M.M., Aghmesheh M., Ow K.T., Rohde P.R., Barrow K.D., Russell P.J. 2000; Beta-human chorionic gonadotropin in semen: a marker for early detection of prostate cancer? Mol. Urol. 4: 421-427.

Davies, S. 2001; Ectopic expression of glycoprotein hormones and their receptors by urogenital cancers. University of London PhD thesis.

de Bruijn H.W., ten Hoor K.A., Krans M., van der Zee A.G. 1997; Rising serum values of β-subunit human chorionic gonadotrophin (hCG) in patients with progressive vulvar carcinomas. Br. J. Cancer 75: 1217-1218.

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.

Devi G.R., Oldenkamp J.R., London C.A., Iversen P.L. 2002; Inhibition of human chorionic gonadotropin beta-subunit modulates the mitogenic effect of c-myc in human prostate cancer cells. Prostate 53: 200-210.

Demir R., Kayisli U.A., Seval Y., Celik-Ozenci C., Korgun ET., Demir-Weusten A.Y., Huppertz B. 2004; Sequential expression of VEGF and its receptors in human placental villi during very early pregnancy: differences between placental vasculogenesis and angiogenesis. Placenta. 25: 560-572.

Dirnhofer S., Koessler P., Ensinger C., Feichtinger H., Madersbacher S., Berger P. 1998; Production of trophoblastic hormones by transitional cell carcinoma of the bladder: association to tumor stage and grade. Hum. Pathol. 29: 377-382.

Dirnhofer S., Freund M., Rogatsch H., Krabichler S., Berger P. 2000; Selective expression of trophoblastic hormones by lung carcinoma: neurendocrine tumors exclusively produce human chorionic gonadotropin β-subunit (hCGβ). Hum Pathol. 31: 966-972.

Djewitzi W. Über einen Fall von Chorionepitheliom der Harnblase. Virchows Arch. Path. Anat. 178: 451-464, 1904.

ACCEPTED MANUSCRIPT

Dobrowolski Z.F., Byrska B., Dolezal M. 1994; Prognostic value of β human chorionic gonadotrophin in blood serum of patients with urinary bladder tumors. Int. Urol. Nephrol. 26: 301-306.

El-Ahmady O., Halim A.B., Mansour O., Salman T., El-Din AG., Walker R.P. 1996; Urinary gonadotropin peptide (UGP) in Egyptian patients with benign and advanced malignant urological disease. Br. J. Cancer 73: 1486-1490.

Eder I.E., Stenzl A., Hobisch A., Cronauer M.V. Bartsch G., Klocker H. 1996; Transforming growth factors-β1 and β2 in serum and urine from patients with bladder carcinoma. J. Urol. 157: 953-957.

Eder I.E., Stenzl A., Hobisch A., Cronauer M.V., Bartsch G., Klocker H. 1997; Expression of transforming growth factors-β1, β2 and β3 in human bladder carcinomas. Br. J. Cancer 75: 1753-1760.

Fabisiewicz A., Kulik J., Kober P., Brewczynska E., Pienkowski T., Siedlecki J.A. 2004; Detection of circulating breast cancer cells in peripheral blood by a two-marker reverse transcriptase-polymerase chain reaction assay. Acta. Biochim. Pol. 51: 747-755.

Fiddes J.C., Talmage K. 1984; Structure, expression and evolution of the genes for the human glycoprotein hormones. Recent Progress in Hormone Research. 40: 43-74.

Fujii D. 1986; Transforming growth factor β gene maps to human chromosome 19 long arm and to mouse chromosome 7. Somatic Cell and Mol. Genet. 12: 281-288.

Gebauer G., Fehm T., Beck E.P., Berkholz A., Licht P., Jager W. 2003; Cytotoxic effect of conjugates of doxorubicin and human chorionic gonadotropin (hCG) in breast cancer cells. Breast Cancer Res. Treat. 77: 125-131.

Geissler M., Wands G., Gesien A., de la Monte S., Bellet D., Wands J.R. 1997; Genetic immunization with the free human chorionic gonadotropin beta subunit elicits cytotoxic T lymphocyte responses and protects against tumor formation in mice. Lab Invest. 76: 859-871.

Gillott D.J., lles R.K., Chard T. 1996; The effects of β-human chorionic gonadotrophin on the *in vitro* growth of bladder cancer cell lines. Br. J. Cancer 73: 323-326.

Giovangrandi Y., Parfait B., Asheuer M., Olivi M., Lidereau R., Vidaud M., Bieche I. 2001; Analysis of the human CGB/LHB gene cluster in breast tumors by real-time quantitative RT-PCR assays. Cancer Lett. 168: 93-100.

Grossmann M., Hoermann R., Gocze P.M., Ott M., Berger P., Mann K. 1995; Measurement of human chorionic gonadotrophin-related immunoreactivity in serum, ascites and tumor cysts of patients with gynaecologic malignancies. Eur. J. Clin. Invest. 25: 867-873.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Guo S., Russo I.H., Lareef M.H., Russo J. 2004; Effect of human chorionic gonadotropin in the gene expression profile of MCF-7 cells. Int. J. Oncol. 24: 399-407.

Halim A-B., El-Ahmady O., Hamza S., Aboul-Ela M., Oehr P. 1995; Urinary β-hCG in benign and malignant urinary tract disease. Disease Markers 12**:** 109-115.

Hansel W, Enright F, Leuschner C. 2007; Destruction of breast cancers and their metastases by lytic peptide conjugates in vitro and in vivo. Mol Cell Endocrinol. 260-262:183-9.

He L.Z., Ramakrishna V., Connolly J.E., Wang X.T., Smith P.A., Jones C.L., Valkova-Valchanova M., Arunakumari A., Treml J.F., Goldstein J., Wallace P.K., Keler T., Endres M.J. 2004; A novel human cancer vaccine elicits cellular responses to the tumor-associated antigen, human chorionic gonadotropin beta. Clin. Cancer Res. 10: 1920-1927.

Hedstrom J., Grenman R., Ramsey H., Finne P., Lundin J., Haglund C., Alfthan H., Stenman U.H. 1999; Concentration of free hCGβ subunit in serum as a prognostic marker for squamous-cell carcinoma of the oral cavity and oropharynx. Int. J. Cancer 84: 525-528.

Herr F., Baal N., Reisinger K., Lorenz A., McKinnon T., Preissner K.T., Zygmunt M. 2007; HCG in the regulation of placental angiogenesis. Results of an in vitro study. Placenta. Suppl A:S85-93.

Hoon D.S., Sarantou T., Doi F., Chi D.D., Kuo C., Conrad A.J., Schmid P., Turner R., Guiliano A. 1996; Detection of metastatic breast cancer by beta-hCG polymerase chain reaction. Int. J. Cancer 69: 369-374.

Hotakainen K., Ljunberg B., Paju A., Rasmusen T., Althan H., Stenman U.H. 2002a; The free beta subunit of human chorionic gonadotrophin as a prognostic factor in renal cell carcinoma. Br. J. Cancer 86: 185-189.

Hotakainen K, Haglund C, Paju A, Nordling S, Alfthan H, Rintala E, Stenman UH. 2002b; Chorionic gonadotropin beta-subunit and core fragment in bladder cancer: mRNA and protein expression in urine, serum and tissue. Eur Urol. 41:677-85.

Hotakainen K, Lintula S, Jarvinen R, Paju A, Stenman J, Rintala E, Stenman UH. 2007; Overexpression of human chorionic gonadotropin beta genes 3, 5 and 8 in tumor tissue and urinary cells of bladder cancer patients. Tumour Biol. 28:52-6.

Hu YL, Lei ZM, Huang ZH, Rao CV. 1999; Determinants of Transcription of the Chorionic Gonadotropin /Luteinizing Hormone Receptor Gene in Human Breast Cells. Breast J. 5:186-193.

Hu XC, Chow LW. 2001; Detection of circulating breast cancer cells with

ACCEPTED MANUSCRIPT

multiple-marker RT-PCR assay. Anticancer Res. 21:421-4.

Hu X.C., Loo WT., Chow L.W. 2003; Surgery-related shedding of breast cancer cells as determined by RT-PCR assay. J. Surg. Oncol. 82: 228-232.

Iles R.K., Butler S.A. 1998; Human urothelial carcinomas - A typical disease of the aged: The clinical utility of chorionic gonadotrophin in patient management and future therapy. Exp. Gerontol., 33: 379-391.

Iles R.K., Chard T. 1989; Immunochemical analysis of the human chorionic gonadotrophin-like material secreted by 'normal' and neoplastic urothelial cells. J. Mol. Endo. 2: 107-112.

Iles R.K., Chard T. 1991; Human chorionic gonadotrophin expression by bladder cancers: biological and clinical potential. J. Urol. 145: 453-458.

Iles R.K., Czepulkowski B.H., Young B.D., Chard T. 1989; Amplification and re-arrangement of the β-human chorionic gonadotrophin (β-hCG)-human LH gene cluster is not responsible for the ectopic production of β-hCG by bladder tumor cells. J. Mol. Endocrinol. 2: 113-117.

Iles R.K., Lee C.L., Oliver R.T.D, Chard T. 1990a; Composition of intact hormone and free subunits in the hCG-like material found in serum and urine of patients with carcinoma of the bladder. Clinical Endocrinol. 32: 355-364.

Iles R.K., Persad R., Trivedi M., Sharma K.B., Dickinson A., Smith P., Chard T. 1996; Urinary concentration of human chorionic gonadotrophin and its fragments as a prognostic marker in bladder cancer. Br. J. Urol. 77: 61-69.

Iles, R.K., Purkis, P.E., Whitehead, P.C., Oliver, R.T.D., Leigh, I., Chard, T. 1990b; Expression of β human chorionic gonadotrophin by non-trophoblastic nonendocrine 'normal' and malignant epithelial cells. Br. J. Cancer 61: 663-666.

Ind T., Iles R., Shepherd J., Chard T. 1997; Serum concentrations of cancer antigen 125, placental alkaline phosphatase, cancer-associated serum antigen and free β human chorionic gonadotrophin as prognostic markers for epithelial ovarian cancer. Br. J. Obs. Gyn. 104: 1024-1029.

Jackson A.M., Klonisch T., Lapthorn A.J., Berger P., Isaacs N.W., Delves P.J., Lund T., Roitt I.M. 1996; Identification and selective destruction of shared epitopes in human chorionic gonadotropin beta subunit. J. Reprod. Immunol. 31: 21-36.

Jankowska A, Gunderson SI, Andrusiewicz M, Burczynska B, Szczerba A, Jarmolowski A, Nowak-Markwitz E, Warchol JB. 2008; Reduction of human chorionic gonadotropin beta subunit expression by modified U1 snRNA caused apoptosis in cervical cancer cells. Mol Cancer. ;7:26.

ACCEPTED MANUSCRIPT

Janssens J.P., Verlinden I., Gungor N., Raus J., Michiels L. 2004; Protein biomarkers for breast cancer prevention. Eur. J. Cancer Prev. 13: 307-317.

Janssens JP, Russo J, Russo I, Michiels L, Donders G, Verjans M, Riphagen I, Van den Bossche T, Deleu M, Sieprath P. 2007; Human chorionic gonadotropin (hCG) and prevention of breast cancer. Mol Cell Endocrinol. 269:93-98.

Jiang Y., Zeng F., Xiao C., Liu J. 2003; Expression of beta-human chorionic gonadotropin genes in renal cell cancer and benign renal disease tissues. J. Huazhong Univ. Sci. Technolog. Med. Sci. 23: 291-293.

Keler T, He L, Ramakrishna V, Champion B. 2007; Antibody-targeted vaccines. Oncogene**.** 26:3758-67

Kido A., Mory M., Adachi Y., Yukaya H., Ishida T., Sugimachi K. 1996; Immunohistochemical expression of β-human chorionic gonadotropin in colorectal carcinoma. Surg. Today. 26: 966-970.

Kocdor H, Kocdor MA, Russo J, Snider KE, Vanegas JE, Russo IH, Fernandez SV. 2009;
Human chorionic gonadotropin (hCG) prevents the transformed phenotypes induced by 17 beta-estradiol in human breast epithelial cells. Cell Biol Int.33:1135-1143.

Lapthorn A.J., Harris D.C., Littlejohn A., Lustbader J.W., Canfield R.E., Machin K.J., Morgan F.K., Issacs N.W. 1994; Crystal structure of human chorionic gonadotrophin. Nature 369: 455-461.

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

Louhimo J., Nordling S., Alfthan H., von Boguslawski K., Stenman U.H., Haglund C. 2001; Specific staining of human chorionic gonadotropin β in benign and malignant gastrointestinal tissues with monoclonal antibodies. Histopathology 38: 418-424.

Louhimo J., Carpelan-Holmstrom M., Alfthan H., Stenman U.H., Jarvinen H.J., Haglund C. 2002; Serum hCG □, CA 72-4 and CEA are independent prognostic factors in colorectal cancer. Int. J. Cancer 101: 545-548.

Louhimo J., Alfthan H., Stenman U.H., Haglund C. 2004; Serum hCG b and CA 72-4 are stronger prognostic factors than CEA, CA 19-9 and CA 242 in pancreatic cancer. Oncology 66: 126-131.

ACCEPTED MANUSCRIPT

Lundin M., Nording S., Carpelan-Holmstrom M., Louhimo J., Alfthan H., Stenman U.H., Haglund C. 2000; A comparison of serum and tissue hCG as prognostic markers in colorectal cancer. Anticancer Res. 20: 4949-4951.

Mann R.J., Keri R.A., Nilson J.H. 2003; Consequences of elevated luteinizing hormone on diverse physiological systems: use of the LHbetaCTP transgenic mouse as a model of ovarian hyperstimulation-induced pathophysiology. Recent Prog. Horm. Res. 58: 343-375.

Marcillac I., Cottu P., Theodore C., Terrier-Lacombe M.J., Bellet D., Droz J.P. 1993; Free hCG-β subunit as tumor marker in urothelial cancer. Lancet 341: 1354-1355.

Martin J.E., Jenkins B.J., Zuk R.J., Oliver R.T., Baithun S.I. 1989; Human chorionic gonadotrophin expression and histological findings as predictors of response to radiotherapy in carcinoma of the bladder. Virch. Arch. Path. Anat. 414: 273-277.

Meduri G., Charnaux N., Spyratos F., Hacene K., Loosfelt H., Milgrom E. 2003; Luteinizing hormone receptor status and clinical, pathologic, and prognostic features in patients with breast carcinomas. Cancer. 97: 1810-1816.

Mora J., Gascon N., Tabernero JM., Rodriguez-Espinosa, J., Gonzalez-Sastre, F. 1996; Different hCG assays to measure ectopic hCG secretion in bladder carcinoma patients. Br. J. Cancer 74: 1081-1084.

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

Moutzouris G., Yannapoulos D., Barbatis C., Zaharof A., Theodorou C. 1993; Is β human chorionic gonadotrophin production by transitional cell carcinoma of the bladder a marker of aggressive disease and resistance to radiotherapy? Br. J. Urol. 72: 907-909.

Muller YA, Christinger HW, Keyt BA, de Vos AM. 1997; The crystal structure of vascular endothelial growth factor (VEGF) refined to 1.93 A resolution: multiple copy flexibility and receptor binding. Structure. 5: 1325-1338.

Pectasides D., Bafaloucos D., Antoniou F., Gogou L., Economides N., Varthalitis J., Dimitriades M., Kosmidis P., Athanassiou A. 1996; TPA, TATI, CEA, AFP, β-hCG, PSA, SCC and CA 19-9 for monitoring transitional cell carcinoma of the bladder. Am. J. Clin. Oncol. 19: 271-277.

Piccart-Gebhart M.J., Procter M., Leyland-Jones B., Goldhirsch A., Untch M., Smith I., Gianni L., Baselga J., Bell R., Jackisch C., Cameron D., Dowsett M., Barrios C.H., Steger G., Huang C.S., Andersson M., Inbar M., Lichinitser M., Lang I., Nitz U., Iwata

ACCEPTED MANUSCRIPT

H., Thomssen C., Lohrisch C., Suter T.M., Ruschoff J., Suto T., Greatorex V., Ward C., Straehle C., McFadden E., Dolci M.S., Gelber R.D.; Herceptin Adjuvant (HERA) Trial Study Team. 2005; Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N. Engl. J. Med. 353: 1659-1672.

Pierce J.G., Parsons T.F. 1981; Glycoprotein hormones: structure and function. Ann. Rev. Biochem. 50: 465-495.

Pond-Tor S., Rhodes R.G., Dahlberg P.E., Leith J.T., McMichael J., Dahlberg A.E. 2002; Enhancement of radiosensitivity of the MCF-7 breast cancer cell line with human chorionic gonadotropin. Breast Cancer Res. Treat. 72: 45-51.

Porakishvili N., Chiesa M.D., Chikadze N., Martensen P., Justesen J., Lund T., Delves P.J., Roitt I.M. 2002; Elimination of luteinizing hormone cross-reactive epitopes from human chorionic gonadotropin. Vaccine. 20: 2053-2059.

Rao ChV., Li X., Manna S.K., Lei Z.M., Aggarwal B.B. 2004; Human chorionic gonadotropin decreases proliferation and invasion of breast cancer MCF-7 cells by inhibiting NF-kappaB and AP-1 activation. J. Biol .Chem. 279: 25503-25510.

Reisinger K., Baal N., McKinnon T., Münstedt K., Zygmunt M. 2007; The gonadotropins: tissue-specific angiogenic factors? Mol. Cell. Endocrinol. 269: 65-80.

Romond E.H., Perez E.A., Bryant J., Suman V.J., Geyer C.E. Jr., Davidson N.E., Tan-Chiu E, Martino S., Paik S., Kaufman P.A., Swain S.M., Pisansky T.M., Fehrenbacher L., Kutteh L.A., Vogel V.G., Visscher D.W., Yothers G., Jenkins R.B., Brown A.M., Dakhil S.R., Mamounas E.P., Lingle W.L., Klein P.M., Ingle J.N., Wolmark N. 2005; Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N. Engl. J. Med. 353: 1673-1684.

Rull K, Laan M. 2005; Expression of beta-subunit of HCG genes during normal and failed pregnancy. Hum Reprod. 20:3360-3368.

Russo J. & Russo I . 2004 *Molecular Basis of Breast Cancer: Prevention and Treatment.* Springer-Verlag, Berlin, Heidelberg, New York, ISBN 3-540-00391-6

Ryan RJ, Charlesworth MC, McCormick DJ, Milius RP, Keutmann HT. 1988; The glycoprotein hormones: recent studies of structure-function relationships. FASEB J. 11: 2661-9.

Salhab M., Al Sarakbi W., Mokbel K. 2005; In vitro fertilization and breast cancer risk: a review. Int. J. Fertil. Womens Med. 50: 259-266.

Scholl P.D., Jurco S., Austin J.R. 1997; Ectopic production of β-hCG by a maxillary squamous cell carcinoma. Head & Neck 19: 701-705.

ACCEPTED MANUSCRIPT

Schwarz-Roeger U., Petzoldt B., Waldschmidt R., Walker R.P., Bauknecht T., Kiechle M. 1997; UGP- a tumor marker of gynecologic and breast malignancies? Specificity and sensitivity in pretherapeutic patients and the influence of hormonal substitution on the expression of UGP. Anticancer Res. 17: 3041-3045.

Sheaff M.T., Martin J.E., Badenoch D.F., Baithun S.I. 1996; βhCG as a prognostic marker in adenocarcinoma of the prostate. J. Clin. Path. 49: 329-332.

Slodkowska J., Szturmowicz M., Rudzinski P., Giedronowicz D., Sakowicz A., Androsiuk W., Zakrzewska-Rowinska E. 1998; Expression of CEA and trophoblastic cell markers by lung carcinoma in association with histological characteristics and serum marker levels. Eur. J. Cancer Prev. 7: 51-60.

Span P.N., Thomas C.M., Heuvel J.J., Bosch R.R., Schalken J.A., vd Locht L., Mensink E.J, Sweep C.G. 2002; Analysis of expression of chorionic gonadotrophin transcripts in prostate cancer by quantitative Taqman and a modified molecular beacon RT-PCR. J. Endocrinol. 172: 489-495.

Span P.N., Manders P., Heuvel J.J., Thomas C.M., Bosch R.R., Beex L.V., Sweep C.G. 2003; Molecular beacon reverse transcription-PCR of human chorionic gonadotropin-beta-3, -5, and -8 mRNAs has prognostic value in breast cancer. Clin. Chem. 49: 1074-1080.

Stenman U.H., Alfthan H., Hotakainen K. 2004; Human chorionic gonadotropin in cancer. Clin. Biochem. 37: 549-561.

Sun P.D., Davies D.R. 1995; The cystine-knot growth-factor superfamily. Ann. Rev. Biophys. Biomol. Struct. 24: 269-291.

Syrigos K.N., Fyssas I., Konstandoulakis M.M., Harrington K.J., Papadopoulos S., Milingos N., Peveretos P., Golematis B.C. 1998; Beta human chorionic gonadotropin concentrations in serum of patients with pancreatic adenocarcinoma. Gut 42: 88-91.

Szturmowicz M., Wiatr E., Sakowicz A., Slodkowska K., Filipecki S., Rowinska-Zakrzewska E.R. 1999; The role of human chorionic gonadotropin β subunit elevation in small-cell lung cancer patients. Tumor Biol. 20: 99-104.

Taback B., Chan A.D., Kuo C.T., Bostick P.J., Wang H.J., Giuliano A.E., Hoon D.S. 2001; Detection of occult metastatic breast cancer cells in blood by a multimolecular marker assay: correlation with clinical stage of disease. Cancer Res. 61: 8845-8850.

Talwar G.P. 1999; Vaccines and passive immunological approaches for the control of fertility and hormone-dependent cancers. Immunol. Rev. 171: 173-192.

Tanaka Y, Kuwabara K, Okazaki T, Fujita T, Oizumi I, Kaiho S, Ogata E. 2000; Gonadotropins stimulate growth of MCF-7 human breast cancer cells by promoting

ACCEPTED MANUSCRIPT

intracellular conversion of adrenal androgens to estrogens. Oncology. 59 Suppl 1:19-23

Triozzi PL, Stevens VC. 1999; Human chorionic gonadotropin as a target for cancer vaccines. Oncol Rep. 6: 7-17.

Vaitukaitis J.L., Braunstein G.D., Ross G.T. 1972; A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. Am. J. Obstet. Gynecol. 113: 751-758.

Webb A., Scott-Mackie P., Cunningham D., Norman A., Andreyev J., O'Brien M., Bensted J. 1995; The prognostic value of CEA, β HCG, AFP, CA125, CA19-9 and C-erb B-2, β hCG immunohistochemistry in advanced colorectal cancer. Ann. Oncol. 6: 581-587.

Wu H., Lustbader J.W., Liu Y., Canfield R.E., Hendrickson W.A. 1994; Structure of human chorionic gonadotropin at 2.6 Å resolution from MAD analysis of the selenomethionyl protein. Structure 15: 545-558.

Wachner R, Wittekind C, von Kleist S. 1984; Immunohistological localization of beta-HCG in breast carcinomas. Eur J Cancer Clin Oncol. 20:679-84.

Xie Y.B, Wang H., Segaloff D.L. 1990; Extracellular domain of lutropin/choriogonadotropin receptor expressed in transfected cells binds choriogonadotropin with high affinity. J. Biol. Chem. 265: 21411–21414.

Yi H., Rong Y., Yankai Z., Wentao L., Hongxia Z., Jie W., Rongyue C., Taiming L., Jingjing L. 2006; Improved efficacy of DNA vaccination against breast cancer by boosting with the repeat beta-hCG C-terminal peptide carried by mycobacterial heat-shock protein HSP65. Vaccine 24: 2575-2584.

Yokotani T., Koizumi T., Taniguchi R., Nakagawa T., Isobe T., Yoshimura M., Tsubota H., Hasegawa K., Ohsawa N., Baba S., Yasui H., Nishimura R. 1997; Expression of α and β genes of human chorionic gonadotropin in lung cancer. Int. J. Cancer 71: 539-544.

Zondek B. 1930: Versuch einer biologischen (hormonalen) Diagnostik beim malignen Hodentumor. Chirug. 2: 1072–1080.

Zondek, B. 1935: Hormone des Ovariums und des Hypophysenvorderlappens, Springer, Vienna. pp. 210-248.

Zygmunt M., Herr F., Keller-Schoenwetter S., Kunzi-Rapp K., Münstedt K., Rao C.V., Lang U., Preissner K.T. 2002; Characterization of human chorionic gonadotropin as a novel angiogenic factor. J. Clin. Endocrinol. Metab. 87: 5290-5296.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Yi H, Rong Y, Yankai Z, Wentao L, Hongxia Z, Jie W, Rongyue C, Taiming L, Jingjing L. 2006; Improved efficacy of DNA vaccination against breast cancer by boosting with the repeat beta-hCG C-terminal peptide carried by mycobacterial heat-shock protein HSP65. Vaccine. 24: 2575-2584.

Yves A Muller, Hans W Christinger, Bruce A Keyt, Abraham M de Vos. (1997).Vascular endothelial growth factor: Crystal structure and functional mapping of the kinase domain receptor binding sit*e*. Proc. Natl. Acad. Sci. USA 9: 7192–7197

Table and figure legends

Table 1. Frequency and significance of hCGβ expression by common carcinomas. Case reports have been omitted as they do not add information regarding the frequency of ectopic hCGβ expression. Thirty-seven of 40 reports investigated the frequency of elevated levels of hCGβ (or hCGβcf) in serum or urine and/or detection in tissue sections by immunohistochemistry (IHC). Positive detection ranges from 0% in renal cell cancer to 93% in small cell (SC) lung cancer; ~30% of epithelial cancer patients will have elevated serum levels, 37% elevated urinary levels and 48% of tumors will stain hCGβ positive. Twenty three of the studies specifically investigated cancer prognosis and 20 (87%) report a strong association between hCGβ detection and poor patient prognosis - metastatic spread. Other studies have investigated mRNA levels such as prostate cancer (Daja *et al*., 2000, Span *et al*., 2002) and renal carcinoma (Jiang *et al*., 2003) but many such studies were compromised by high-level hCGβ mRNA detection in normal tissues. mRNA detection of hCGβ detected in breast cancer cells is described in Table 3.

Table 2 - Studies suggesting that hCG has a direct protective effect on breast tissue and anti-cancer activity. Three studies have examined LH/hCG receptor expression by breast tissue; one used such expression to target drug therapy and six studies have demonstrated hCG stimulation of secondary messengers and change in breast cancer cell gene expression as a result.

Table 3 – Studies reporting breast tumor ectopic expression of hCGβ. These studies have demonstrate expression as detected by IHC but the incidence (24%) is lower than the average (~48%, see table 1) and is not reported as associated with prognosis. hCGβ has been targeted as a marker for the detection of shed circulating breast cancer cells and is detected in 36-38% of cases.

Figure 1: Cystine Knot superfamily – Diagrammatic representation of the cystine knot hormones/growth factors grouped by functional family. Functional families in which hCG/free hCGβ may cross-react are expanded in detail.

Figure 2: Comparative shapes of cystine knot dimers. Molecular models of the 3D structure of dimeric TGFβ, VEGF, NGF and a theoretical hCGββ homodimer.

ACCEPTED MANUSCRIPT

Figure 3 hCGβ-LHβ gene cluster ch19q13.32,- Diagrammatic representation of the relative position of *hCGβ* genes *1,2,3,5,7,8* and *LHβ* interspersed with neurotropin pseudogenes- *NTF4, 6, 6a* and *6G.*

Accepted Manuscript

ACCEPTED MANUSCRIPT

Tissue of origin **Author & Date**	**Serum** **hCGβ**	**Urine** **hCGβ/βcf**	**IHC** **hCGβ**	**Prognostic**
Bladder				
Dirnhofer *et al*., *1998*	-	-	35% (*n* =104)	-
Mora *et al*., *1996*	76% (*n* =33)	-	-	-
El-Ahmady *et al*., *1996*	-	73% (*n* =237)	-	-
Pectasides *et al*., *1996*	30% (*n* =76)	-	-	Yes
Iles *et al*., *1996*	-	35% (*n* =52)	-	Yes
Halim *et al*., *1995*	-	60% (*n* =63)	-	-
Lung				
Dirnhofer *et al*., 2000	-	-	9% (*n* =90)	-
Szturmowicz *et al.*, 1999	14% (*n* =85)	-	28% (*n* =85)	Yes
Slodkowska *et al.*, 1998	22% (*n* =92)	-	-	-
Yokotani *et al.*, 1997	-	-	64% (*n* =14)	-
Boucher *et al.*, 1995	-	-	93% (*n* =44)	-
Oral/Facial				
Hedstrom *et al.*, 1999	20% (*n* =59)	-	-	Yes
Bhalang *et al.*, 1999	-	-	64% (*n* =45)	Yes
Scholl *et al.*, 1997	-	-	100% (*n* =1)	-
Breast				
Agnanatis *et al.*, 1992	-	-	56% (*n*=27)	-
Bieche *et al.*, 1998	-	-	49% (*n* =99)	No
Chang *et al.*, 2005	-	-	13% (*n* =334)	-
Schwarz-Roeger *et al.*, 1997	-	19% (*n* = 72)	-	-
Hoon *et al.*, 1996	-	-	80% (*n* =32)	-
Cervical				
Crawford *et al.*, 1998	-	33% (*n* =46)	26% (*n* =46)	Yes
Schwarz-Roeger *et al.*, 1997	-	29% (*n* =49)	-	-
Grossmann *et al.*, 1995	35% (*n* =40)	-	-	-
Li et al., 2008	-	-	78% (*n*=89)	
Ovarian				
Ind *et al.*, 1997	36% (*n* =73)	-	-	Yes
Grossman *et al.*, 1995	41% (*n* =27)	-	-	-
Vartiainen *et al.*, 2008	33% (*n* = 173)	-	-	Yes
Endometrial				
Schwarz-Roeger *et al.*, 1997	-	32% (*n* =25)	-	-
Grossman *et al.*, 1995	30% (*n* =39)	-	-	-
Vulval/Vaginal				
De Bruijn *et al*., 1997	10% (*n* =118)	-	-	Yes
Schwarz-Roeger *et al.*, 1997	-	17% (*n* =12)	-	-
Carter *et al.*, 1995	38% (*n* =50)	-	-	Yes
Colorectal				
Louhimo *et al*., 2002	16% (*n* =204)	-	-	Yes
Lundin *et al*., 2000	17% (*n* =232)	-	22% (*n* =232)	Yes
Kido *et al.*, 1996	-	-	37% (*n* =123)	Yes
Webb *et al.*, 1995	-	-	54% (*n* =377)	Yes
Prostate				
Sheaff *et al.*, 1996	-	-	15% (*n* =80)	Yes
Ottiti (and Iles) et al.,	4% (*n*=104)	10% (*n*=104)	-	No
Pancreas				
Louhimo *et al*., 2004	50% (*n* =160)	-	-	Yes
Louhimo *et al*., 2001	-	-	56% (*n*=107)	Yes
Syrigos *et al.*, 1998	42% (*n* =36)	-	-	Yes
Renal				
Berzal-Cantalejo *et al* ., 2004	-	-	0% (*n*=55)	No
Hotakainen *et al*., 2002	23% (*n* =177)	-	-	Yes
Neuro-Endocrine				
Shah *et al.*, 2008	12% (*n*=360)	-	-	Yes

ACCEPTED MANUSCRIPT

Table 2 -

Reference	Findings
Hu *et al*., 1999	HCG/LH receptor expression much higher in MCF-7 cells than other breast cancer and tissue cell lines
Pond-Tor *et al*., 2002	Treatment with hCG increases MCF-7 breast cancer cells sensitivity to radiotherapy
Gebaur *et al*., 2003	Conjugation of deoxrubicin to hCG provides as specific targeting modality for cytotoxic drug delivery to LH/hCG receptor positive breast cancer cells
Guo *et al*., 2004	hCG significantly alters the gene expression array of breast cancer cell line MCF-7
Carlsen *et al*., 2004	- IHC and ISH detection of LH/hCG receptor in all male breast tissue samples - intense IHC staining in 0/4 normal 5/13 gynecomastia and 4/5 cancer samples
Rao *et al*., 2004	hCG down regulates proliferation and matrigel invasion by MCF-7 by lowering responsiveness of NFkB and AP-1 to stimuli
Janssens *et al*., 2004	Review of potential of recombinant hCG as a protective drug
Meduri *et al*., 2004	160 patient tumors were examined for LH/hCG receptor, 72% stained (strongly) positive. Positive staining was associated with increased metastases- free survival
Jansseens *et al*., 2007	Pilot study of rhCG administered to patients with primary breast cancer. Excised treated tumors had reduced proliferation index and reduced estrogen &progesterone receptor expression. Metastatic breast cancer patients also received rhCG.
Kocdor *et al*., 2009	MCF-10F cells treated with rhCG differentiated and prevented 17 β-estradiol transformation of cells in collagen matrix cultures into solid masses.

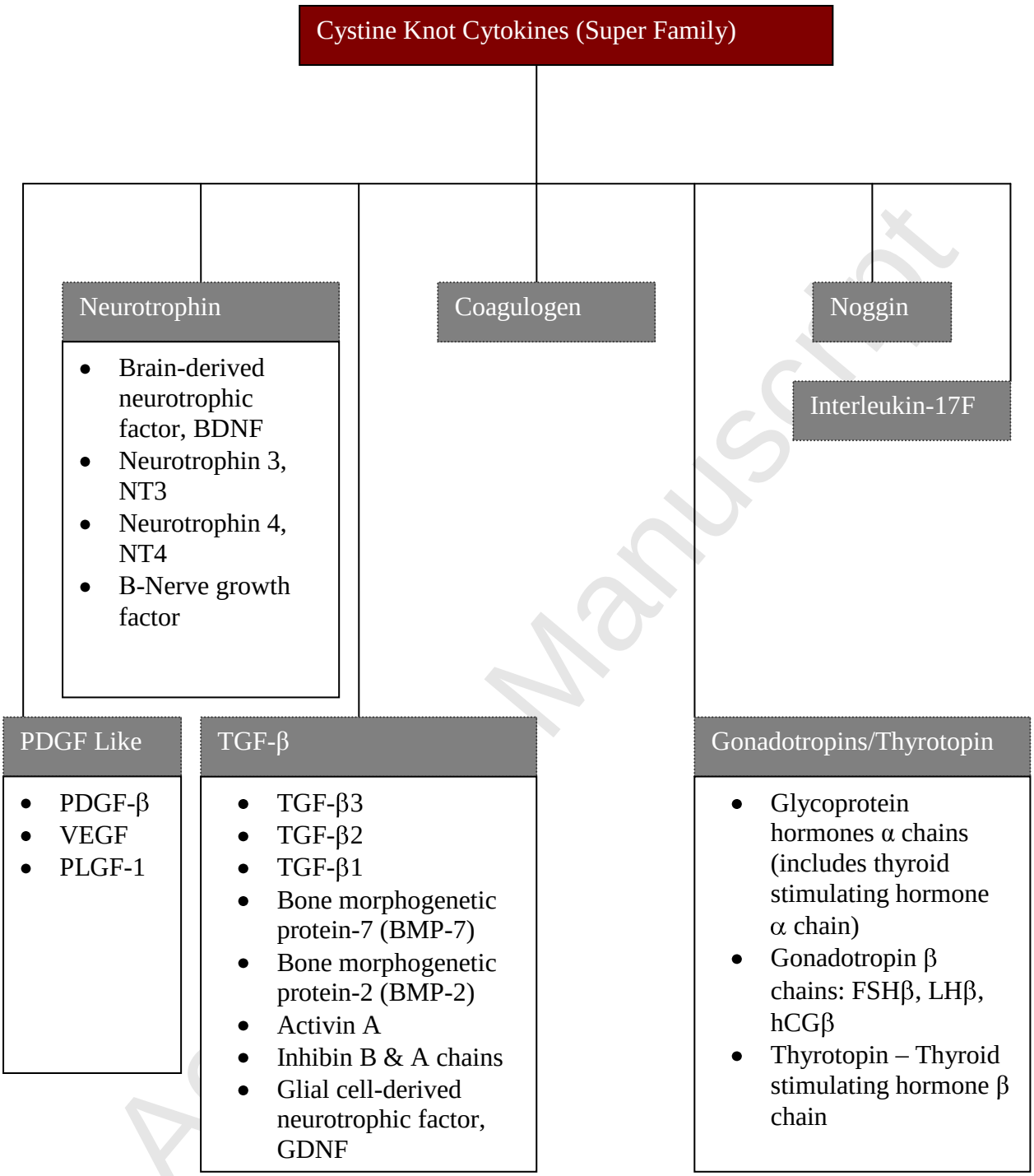
Accepted Manuscript

ACCEPTED MANUSCRIPT

Table 3 -

Reference	Findings
Wachner *et al*., 1984	15/129 tumors stained positive (but only very few per field).
Agnanatis *et al*., 1992.	15/27 tumors stained strongly positive for hCGβ
Taback *et al*., 2001	HCGβ mRNA was detected in 19 of 25 primary breast cancer tissues and 22 of 65 peripheral blood samples from breast cancer patients at diagnosis. Primary breast cancer tumour tissue expressed functional hCGβ gene transcripts but not the mRNA for the alpha subunit Thus, although primary breast cancer also expressed mRNA for the LH/hCG receptor there could not be auto stimulation of this receptor as the alpha-beta heterodimer hCG could not be formed.
Hu & Chow 2001	hCGβ mRNA was detected in 9/72 breast cancer patients peripheral blood cancer cells and detection correlated with stage of disease.
Tanaka *et al*., 2000	Although hCG did not directly stimulate MCF-7 breast cancer cell growth, it stimulates expression of aromatase enzymes such that DHEA is converted to estradiol, and hence stimulates estrogen dependant MCF-7 cell growth.
Span et al 2003	RT-PCR Analysis of hCGβ expression of 129 patients breast cancer tissue found high hCGβ mRNA levels in 65. High-level expression correlated with lower progression free survival, and was an independent marker to nodal status.
Hu *et al*., 2003	Circulating cancer cells were isolated from blood samples taken from 49 patients. hCGβ mRNA expression transcripts were detected in 6 pre-operatively and 13 immediately post-operatively. Expression did not correlate with recurrence and metastases.
Fabisiewicz *et al*., 2004	RT-PCR detects hCGß mRNA in peripheral blood circulating tumour cells of 21/55 breast cancer patients (stage 1-11 N0-N1)
Chang *et al*., 2005	44/334 tumors stained positive for hCGβ more common in apocrine breast cancers (4/5 positive)

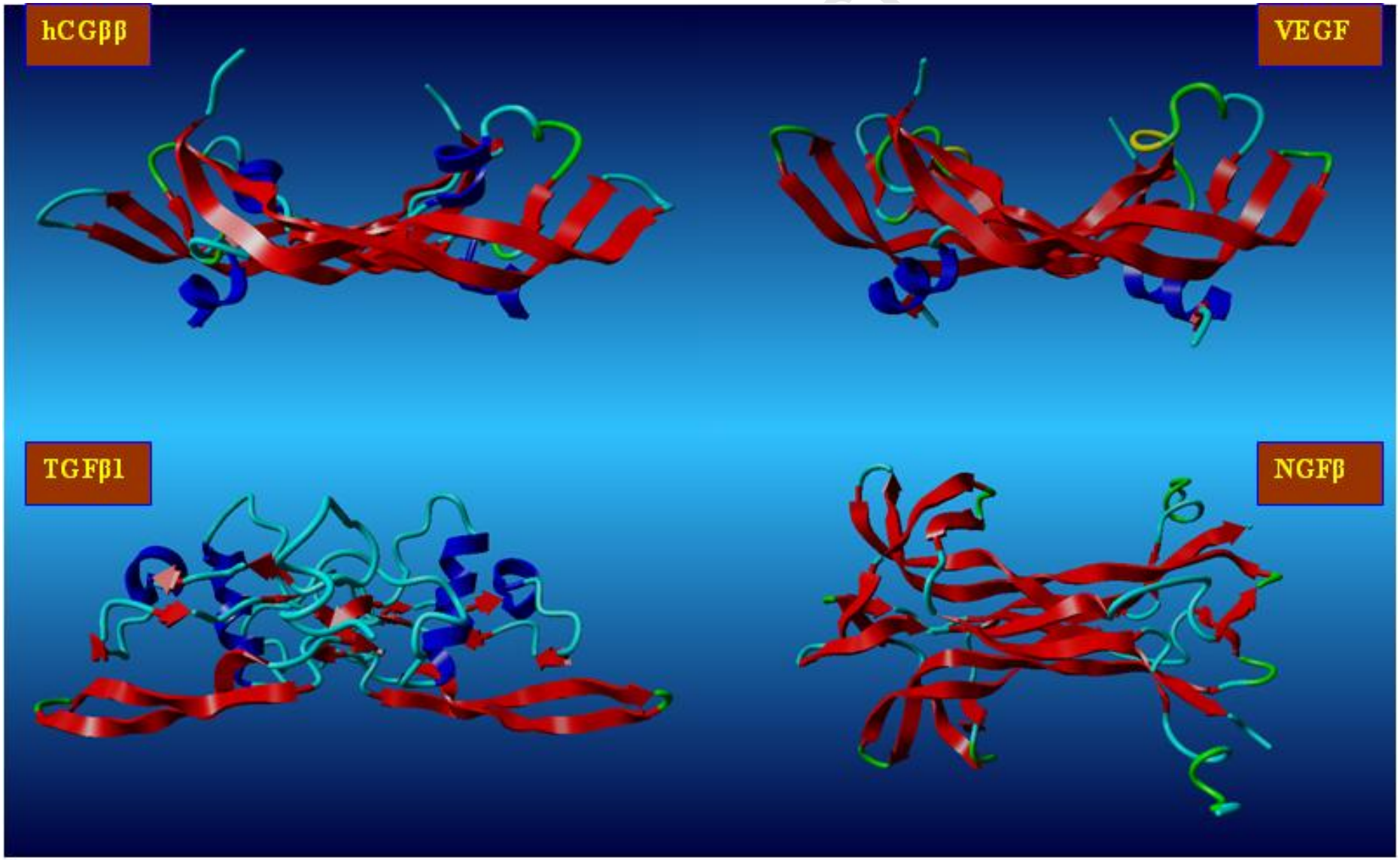
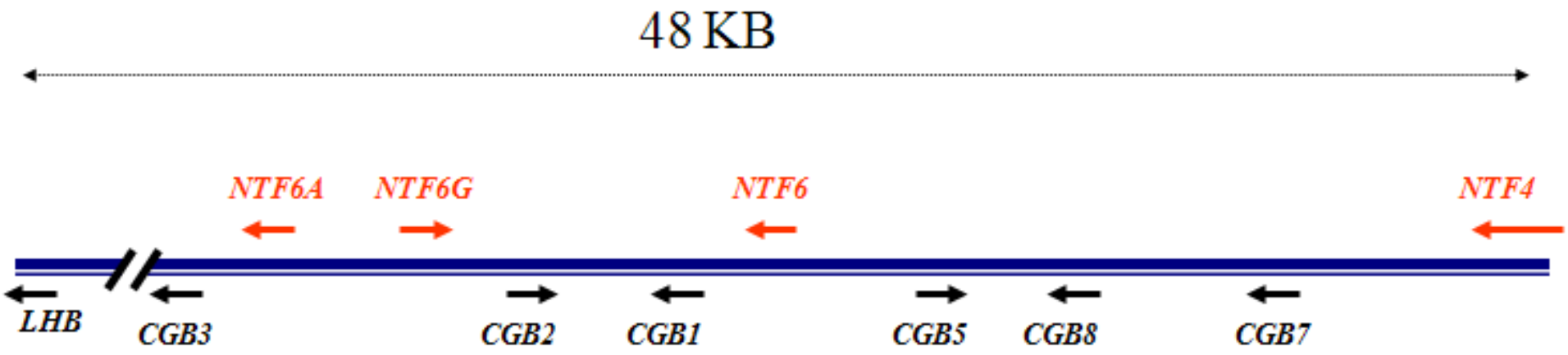
ACCEPTED MANUSCRIPT



Accepted Manuscript

Figure

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT