Aberrant trophoblastic differentiation in human cancer: An emerging novel therapeutic target (Review)

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Abstract. Various types of human cancer may develop aberrant trophoblastic differentiation, including histological changes and altered expression of β-human chorionic gonadotropin (β-hCG). Aberrant trophoblastic differentiation in epithelial cancer is usually associated with poor differentiation, tumor metastasis, unfavorable prognosis and treatment resistance. Since β-hCG-targeting vaccines have failed in an early phase II trial, it is crucial to obtain a better understanding of the molecular pathogenesis of trophoblastic differentiation in human cancer. The present review summarizes the clinical and translational research on this topic with the aim of accelerating the development of an effective targeted therapy. Ectopic expression of β-hCG promotes proliferation, migration, invasion, vasculogenesis and epithelial-mesenchymal transition (EMT) *in vitro*, and enhances metastatic and tumorigenic capabilities *in vivo*. Signaling cascades modulated by β-hCG include the TGF-β receptor pathway, EMT-related pathways, the c-MET receptor tyrosine kinase and mitogen-activated protein kinase/ERK pathways, and the SMAD2/4 pathway. Taken together, these findings indicated that TGF-β receptors, c-MET and ERK1/2 are potential therapeutic targets. Nevertheless, further investigation on the molecular basis of aberrant trophoblastic differentiation is mandatory to improve the design of precision therapy for this aggressive type of human cancer.

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Contents

1. Introduction

Epithelial carcinoma occasionally contains elements reminiscent of trophoblastic differentiation, such as syncytiotrophoblasts and areas resembling choriocarcinoma (1). Immunohistochemical studies have revealed the expression of β-human chorionic gonadotropin (β-hCG) and other trophoblastic hormones in syncytiotrophoblast-like and cytotrophoblast-like cells (1,2). Moreover, non-giant cancer cells in humans may express hCG in primary carcinoma of the bladder, upper urinary tract, prostate, colon, lung, testis and gynecological malignancies. Thus, the expression of β-hCG suggests aberrant differentiation of cancer cells toward a gestational trophoblastic phenotype (1-6). Ectopic expression of β-hCG is usually associated with poor clinicopathological indicators and unfavorable clinical outcomes (3,4,7-10). In previous analyses using β-hCG as a biomarker, carcinoma with trophoblastic differentiation was revealed to be significantly associated with poor differentiation (2,4,10-15), advanced tumor stage (2,4,9,10,13), early hematogenous spread (7,10), chemoresistance (16,17), radioresistance (3,8,10,18) and shorter disease-specific overall survival (4,8). The most well-known example of trophoblastic differentiation in human cancer is observed in urothelial carcinoma (UC) with trophoblastic differentiation (UCTD) (4).

In vitro studies have indicated that overexpression of β-hCG promotes the migration and invasion of ovarian cancer cells, and facilitates their metastasis into peritoneal xenografts (19). Moreover, upregulation of β-hCG can promote the transition of cancer cells from an epithelial phenotype with relevant biomarkers to a loosely adherent, motile phenotype with mesenchymal markers [epithelial-mesenchymal transition (EMT)], and can reduce their adhesive ability (19,20). In a mouse model of human colorectal cancer (CRC), β-hCG-overexpressing cells were shown to exhibit increased invasiveness, migratory ability and metastatic potential (20).

EMT is the dynamic transition of cells from an epithelial to a mesenchymal phenotype. This transition has been identified as the main driver of tumor progression and metastasis (21). During normal placentation, three main trophoblast populations exist: Cytotrophoblast stem cells and their differentiated derivatives, syncytiotrophoblasts and extravillous cytotrophoblasts. Syncytiotrophoblasts primarily exhibit the epithelial phenotype, whereas extravillous cytotrophoblasts undergo EMT, initially forming multilayered cell columns and then (in humans) deeply infiltrating the maternal decidual stroma and blood vessels (22). In epithelial carcinoma, EMT initiates the dissociation of cancer cells from primary tumors, and these cells subsequently migrate and disseminate to distant sites. To the best of our knowledge, the molecular mechanisms underlying trophoblastic differentiation in nongestational human cancer remain unclear. In an autopsy study, only tumor cells with trophoblastic differentiation, but not UC cells, were identified at multiple metastatic sites after treatment with gemcitabine and oxaliplatin (7), supporting the potential resistance of UCTD to standard chemotherapy. Thus, identification of the mechanisms underlying β-hCG-mediated EMT may facilitate the development of targeted therapy against tumor progression through aberrant trophoblastic differentiation. The present review summarizes the literature on the molecular pathogenesis of β-hCG in nontrophoblastic human cancer, with the aim of accelerating the development of targeted therapies for trophoblastic differentiation in human cancer.

2. Histopathology of trophoblastic differentiation and diagnosis

There is a spectrum of trophoblastic differentiation in human cancer, from syncytiotrophoblasts, areas resembling choriocarcinoma, pure choriocarcinoma to other trophoblastic tumors, such as epithelioid trophoblastic tumors (8). The tumor cells exhibit hyperchromatic trophoblastic-like cells with deep eosinophilic cytoplasm that usually appear in the periphery of the tumor nests or infiltrate the interstitium. Tumors may also have scattered multinucleate giant cells and well-defined syncytiotrophoblastic cells or syncytiotrophoblastic cells that wrap around mononuclear tumor cells that resemble cytotrophoblasts (4). In contrast with the inner mass of conventional carcinoma cells, which have pale to eosinophilic cytoplasm, the architecture is similar to the normal anatomical relationship in chorionic villi. Only tumors showing definite immunostaining for β-hCG are diagnosed as trophoblastic differentiation phenotype of nongestational carcinoma (4,8).

3. Trophoblastic differentiation in UC

As per the current classification criteria outlined by the World Health Organization, UCTD includes UC with giant cells resembling syncytiotrophoblastic giant cells and, rarely, those that are indistinguishable from choriocarcinoma (23). To the best of our knowledge, the incidence of UCTD has not been investigated thoroughly. In our recent cohort study, UCTD was detected in 47 of 859 patients (5.5%) and 65 of 635 patients (10.2%) with bladder and upper urinary tract UC, respectively (4). The incidence rate was significantly lower than that (19-36%) reported in small-scale studies (1,2,24). This inconsistency in incidence rate may be because of our strict inclusion criteria regarding trophoblastic histological features compared with the b-hCG immunostaining data used in earlier studies. Our previous study also demonstrated that β-hCG can be expressed in not only UC with syncytiotrophoblast-like and choriocarcinoma-like features, but also in conventional UC (8). Few studies have investigated the expression of other trophoblastic hormones, such as human placental lactogen (hPL), pregnancy-specific β-1-glycoprotein and placental alkaline phosphatase (24). By contrast, the expression of pituitary hormones with structures similar to that of hCG, such as luteinizing hormone (LH), follicle- stimulating hormone (FSH) and growth hormone, was not found in UCTD (2).

4. Clinical significance of trophoblastic differentiation in UC

UCTD in the bladder is usually detected as nonpapillary, multiple and large (>3 cm) tumors with muscle-invasion and nodal-metastasis at diagnosis (4). In addition, the risks

of recurrence, progression and mortality are significantly higher than conventional UC (1,4). Regarding immunohistochemistry, the nonfocal pattern of b-hCG expression is a key predictor of poor prognosis (4). The aforementioned findings corroborate those of other studies indicating the associations of trophoblastic differentiation, either of syncytiotrophoblasts or b-hCG-positive cells, with higher grades of UC and advanced stages of disease (1,8,24). Notably, elevated levels of b-hCG in the serum may be detected in 20-76% of the total number of patients with advanced-stage disease or metastasis (23,25-28). Several studies have reported the potential of b-hCG as a biomarker of radioresistance and chemoresistance (3,7,18); however, contradictory findings have also been reported (23,25,27).

5. Trophoblastic differentiation in nonurothelial carcinoma and its clinical significance

In nonurothelial carcinoma, trophoblastic differentiation is frequently observed in gestational trophoblastic tumors and ovarian germ cell tumors. In addition, sporadic cases of trophoblastic differentiation have been reported in oral cavity, head and neck, lung, stomach, colorectum, prostate, breast and gynecological tract carcinoma (5,9-13,16,20,29-48).

In a study on squamous cell carcinoma of the oral cavity, β-hCG expression (range, 0.5-5%) was detected in 29 of 45 patients (64%) with oral cancer (11); β-hCG expression had a positive association with tumor differentiation. Furthermore, β-hCG expression has been observed in various histological subtypes of lung cancer, including neuroendocrine carcinoma, squamous cell carcinoma, adenocarcinoma and giant cell carcinoma (12,16,49). Trophoblastic hormone immunoreactivity was previously detected in 31% (28/90) of all lung carcinoma cases, regardless of histological differentiation (12).

Although β-hCG has procarcinogenic activities in other types of carcinoma, its role in breast cancer remains controversial. This hormone reportedly inhibits the proliferation and induces the differentiation of human breast cancer cells *in vitro* (50). Paradoxically, a recent study revealed enhanced proliferation and poor differentiation of β-hCG-expressing breast cancer cells, which translated into higher colonization and invasion abilities of these cells (51). In breast cancer cells, the *BRCA1* mutation reportedly promotes β-hCG-mediated tumorigenesis through TGF-βRII signaling (52).

A previous study showed that β-hCG-producing cells can be found in the normal gastric mucosa, particularly the pylorus (31). β-hCG expression has been detected in 6.0-8.2% of all cases of gastric carcinoma, and the rate is even as high as 53% in some reports (32,33). In general, the presence of β-hCG-positive cells or an elevated level of β-hCG in the serum is associated with poor differentiation, adverse prognosis and advanced tumor stage (9); however, contradictory findings have been reported (34).

In ovarian epithelial cancer, elevated levels of serum β-hCG may be detected in both benign (27.6%) and malignant (67%) neoplasms (13), leading to false-positive pregnancy test results (53). Immunohistochemical expression of β-hCG tends to be higher in intermediate- to high-grade ovarian tumors compared with in low-grade tumors; however, the expression does not vary across the histological subtypes of ovarian cancer. Although a study reported higher expression rates of β-hCG in stage III (Federation of Gynecology and Obstetrics) ovarian mucinous carcinomas than in stage I carcinoma, no prominent association was discovered between β-hCG expression and overall survival (13). Regarding molecular mechanisms, β-hCG overexpression reportedly promotes the transformation and tumorigenesis of human ovarian epithelial cells (54). The association of trophoblastic differentiation with high-grade cancer and disease progression has also been observed in endometrial cancer (5,48).

In summary, β-hCG expression is a well-documented phenomenon in nongestational carcinoma of different organs, and may even be observed in some normal tissues and benign tumors. In most cases, the trophoblastic phenotype is associated with poor differentiation, advanced tumor stage and poor prognosis.

6. Prognostic impact of trophoblastic differentiation in human cancer

In terms of prognostic implications, patients with carcinoma showing aberrant trophoblastic differentiation have been reported to have unfavorable clinical outcomes (3-5,7-10,16). Specifically, the phenotype has been associated with early hematogenous spread (7,10,15), higher risk of chemoresistance (16,17) or radioresistance (3,8,10,18), and shorter disease-specific overall survival. Using β-hCG as a biomarker, our recent study revealed a higher risk of recurrence ($P=0.005$), progression ($P<0.0001$) and patient death ($P<0.0001$) for UCTD than for traditional, high-grade UC of the bladder (4). Notably, patients with UCTD and with circumferential, infiltrative or diffuse patterns of β-hCG expression have been reported to have poorer disease-specific overall survival than those with focal β-hCG expression.

In addition to expression in primary tumors, elevated β-hCG serum levels have been observed in sporadic cases of carcinoma with trophoblastic differentiation in UC (1,7,14), squamous cell anal cancer (30), gastric cancer (9), non-small cell carcinoma of the lung (36), pulmonary pleomorphic carcinoma (46), endometrial adenocarcinoma (5,48), lymphoepithelioma-like carcinoma and squamous cell carcinoma of the cervix (55). However, the actual incidence of abnormal laboratory results for this entity has not been thoroughly examined. In our experience, most UCTDs showing a focal pattern of β-hCG expression have normal serum levels (unpublished data). Nevertheless, the levels of serum β-hCG appear to change with treatment (5-7,30,48) and are associated with clinical outcome (5,9,16). Specifically, a serum β-HCG level ≥4 IU/l prior to chemotherapy has been reported to be a significant prognostic factor for patients with advanced gastric cancer (hazard ratio 1.7; 95% confidence interval 2.8-1.1) (9). Serum β-hCG elevation (≥5 mIU/ml) in patients with non-small cell lung cancer showing trophoblastic differentiation is also significantly associated with chemoresistance (16). Taken together, these findings indicated that serum β-hCG measurement may have potential as a marker of clinical response or prognosis, and thus should be applied as a potential biomarker during follow-up after surgery.

7. hCG: Gene, protein and structure

The α-subunit of hCG is encoded by a single gene (*CGA*) on chromosome 6q21.1-23 (56), whereas the β-subunit is encoded by six nonallelic genes (*CGB1*, *CGB2*, *CGB3*, *CGB5*, *CGB7* and *CGB8*) on chromosome 19q13.3 (57). *CGB4*, which is adjacent to the aforementioned *CGB* cluster, encodes the β-subunit of LH. *CGB6* is an allelic variant of *CGB7*, whereas *CGB9* is an allelic variant of *CGB3*. To the best of our knowledge, the functions of *CGB1* and *CGB2* remain unknown; these genes may be pseudogenes (57). The expression of CGB genes is upregulated to some extent in the first trimester of pregnancy, which suggests a role in implantation (58). A protein encoded by type I genes (*CGB6* and *CGB7*) contains an alanine residue at position 117, whereas β-hCG, which is encoded by type II genes (*CGB3*, *CGB9*, *CGB5* and *CGB8*), contains an aspartic acid residue at this position. The effect of this heterogeneity on the function and immunoreactivity of β-hCG remains unknown. Type I genes are expressed primarily in benign nontrophoblastic tissues, whereas type II genes are expressed in trophoblastic and malignant tissues (59).

The hormone hCG comprises 237 amino acids and belongs to the glycoprotein hormone family, which includes LH, thyroid-stimulating hormone (TSH) and FSH. The protein members of the aforementioned family are heterodimers comprising α- and β-subunits. The α-subunit contains 92 amino acids and is common across all family members. By contrast, the β-subunit of hCG exhibits varying degrees of homology with other family members (LH, 80-85%; FSH, 36%; TSH, 46%) (60-62). The homology between hCG and LH indicates their common biological function; both bind to the same receptor, the LH/hCG receptor (63). By contrast, FSH and TSH bind to structurally similar but distinct receptors. The β-subunit of hCG contains 145 amino acids, whereas that of LH contains 121 amino acids; this difference originates from a 24-amino-acid-long extension in hCG, known as the C-terminal peptide (61,64).

The α-subunit of hCG comprises two N-linked oligosaccharides (linked to the N atom of asparagine), whereas its β-subunit comprises two N-linked oligosaccharides and four O-linked oligosaccharides (linked to the O atom of serine) (65). The O-linked and N-linked oligosaccharides contain saccharides ranging from trisaccharides to pentasaccharides, and exhibit monoantennary to triantennary structures (66). Posttranslationally modified hCG variants are complex and have three dimeric isoforms: Regular hCG, hyperglycosylated hCG (hCG-H) and sulfated hCG (hCG-S) (62,67). hCG-H is a glycoprotein with excessive branching and complex hCG oligosaccharide side chains. Although hCG and hCG-H share an amino acid sequence, they are distinct glycoproteins with completely different oligosaccharide structures (66). The levels of hCG-H are high in early pregnancy (68) and show a higher trend in malignant diseases than in normal pregnancy. Various aberrant glycosylations have been detected in tumor-derived hCG (69), which has led to the generation of a diverse molecular weight spectra. In one study, the average molecular weight of hCG was determined to be 37,500 Da through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (70); the molecular weights of α-hCG and β-hCG were 14,000 and 23,500 Da, respectively.

hCG-S originates from the pituitary gland and appears to mimic the activities of LH during the menstrual cycle; LH stimulates ovarian granulosa and theca cells to produce relevant hormones, and facilitates follicular growth, luteinization and ovulation (62,67,71). Both hCG and hCG-S act through the LH/hCG receptor, whereas hCG-H acts through the TGF-β receptor as an autocrine and paracrine factor (62).

Trophoblasts represent a key source of hCG. Syncytiotrophoblasts secrete hCG, whereas cytotrophoblasts secrete hCG-H. Cole *et al* (72) reported the following five common forms of hCG: hCG, hCG-H, hCG-S, free β-hCG and free β-hCG-H (72). They classified these proteins into group 1 (proteins produced by placental syncytiotrophoblasts and pituitary gonadotropes) and group 2 (proteins produced by placental cytotrophoblasts and human cancer cells). Group 1 proteins are hormones that act through the hCG/LH receptor, and are central to the menstrual cycle and pregnancy, whereas group 2 proteins perform autocrine functions by antagonizing the TGF-β receptor and are critical in advanced malignancies (67,72).

Structurally, hCG can be classified as intact hCG, α-hCG, β-hCG, partially degraded or nicked hCG and β-hCG, and a β-core fragment (73); the International Federation of Clinical Chemistry has approved this classification.

8. Regulation of hCG expression

The expression and production of hCG may be regulated by various hormones [corticosteroids, progesterone and gonadotropin-releasing hormone (GnRH)], growth factors (epidermal growth factor, placental growth hormone, leukemia inhibitory factor and vascular endothelial growth factor), cytokines (interleukin-6 and tumor necrosis factor-α), ligands of peroxisome proliferator-activated receptors (PPARs), and homeobox genes (62,74-77).

Levels of α-hCG and β-hCG expression vary in normal placenta, with the α/β ratio ranging from 1.7 in the first trimester, to 12 once the pregnancy has reached full term (78). Therefore, the regulatory mechanisms of α-hCG and β-hCG expression may be different. Knowledge regarding the pathways involved in the upregulation of *hCG* genes remains limited. Cyclic adenosine monophosphate (cAMP) appears to regulate the transcription of the α- and β-subunits of hCG in the placenta and choriocarcinoma (79,80) by regulating the 5' cAMP response element (CRE) enhancers of the promoters of both genes through different mechanisms (81,82).

α-hCG. The promoter of the α-subunit gene contains the following five regulatory elements: A trophoblast-specific element (TSE), an α-activating element (αACT), a tandem or duplicated CRE, a junctional regulatory element (JRE) and a CCAAT box. These elements have been categorized into the following three domains: The upstream regulatory domain (URE; TSE and αACT), tandem CRE and the downstream regulatory domain (JRE and CCAAT box) (83-88). Tissue-specific expression of α-hCG has been demonstrated in trophoblasts on the basis of limited or specific tissue distribution of the binding proteins and regulation of the aforementioned elements (88).

The exact locations of αACT and TSE overlap slightly; the sequence of αACT between -161 and -142 overlaps that of TSE between -182 and -159 (89). Furthermore, two regulatory sequences can be noted within this region: A downstream domain located between -172 and -151 and an upstream domain located between -177 and -156 (86). This structure indicates that these regions are activated by at least two types of binding proteins that may be specifically expressed by either pituitary or placental cells (90).

The tandem CRE is responsible for the binding of CRE-binding proteins (CREBs), which belong to the basic region leucine zipper family of proteins. In villous trophoblasts, activating transcription factor (ATF)-1 is more extensively involved in the binding of CRE and, to some extent, CREB-1, than the other members of the ATF/CREB family, such as ATF-2, ATF-3, ATF-4 and CREB-2 (81). In humans, CREB binding may be dependent on URE binding (89). URE-1/αACT binds to members of the ubiquitous GATA family of DNA-binding proteins (91), whereas URE-2/TSE binds to TSE-binding proteins (TSEBs) (92).

β-hCG. The expression of *CGB* among patients is heterogeneous, and the magnitude of expression has varied across studies, possibly because they focused on various pregnancy trimesters (93,94). *CGB* expression also varies across tumors and normal tissues. For example, the expression levels of type II genes (*CGB3*, *CGB9*, *CGB5* and *CGB8*) are higher in bladder tumors than in the normal urothelium (95).

Similar to the α-hCG promoter, the β-hCG promoter comprises a tandem CRE (two repeated CREs), where c-Jun suppresses β-hCG expression (96). At least two additional TSE elements have been reported to cluster in the regulatory region of *CGB5*. The genes encoding the α- and β-subunits of hCG may be coordinately regulated by TSEBs (92). Other transcription factors involved in *CGB* expression include ETS protooncogene 2 (ETS2), activating protein 2 (AP2), promoter selective transcription factor (SP)1, SP3, octamer-binding transcription factor (OCT)3/OCT4, PPARγ, P53 and metastasis-associated protein 3 (MTA3) (97-103).

In human choriocarcinoma and murine cells, ETS2 enhances the transcription of *CGB5* through activation of the rat sarcoma virus/mitogen-activated protein kinase pathway, and the primary effects of cAMP on the β-hCG promoter are mediated through the proximal ETS2 enhancer (97). AP2, SP1 and SP3 are the key regulators of basal *CGB* transcription in placental trophoblast cells (103,104). AP2 and SP1 play distinct roles in the regulation of basal activity and cAMP-responsiveness of the β-hCG promoter (105), whereas SP3 suppresses its basal transcription by inhibiting SP1 (105). The expression levels of *CGB3*-*CGB9* have been reported to be considerably higher in ovarian cancer cells than in healthy ovarian cells (103). *CGB1* and *CGB2* transcripts have been detected in 20% of all ovarian cancer tissue sample, but not in control samples. This may have resulted from demethylation of *CGB* promoter regions, an increased level of transcription factor AP2 (TFAP2)-α, and a decreased level of SP3 in ovarian tumors (103). OCT3 and OCT4 are essential for maintaining embryonic cells in an undifferentiated state; these transcription factors may silence hCG expression in choriocarcinoma cells (99).

The treatment of trophoblasts with PPARγ or retinoid X receptor (RXR)α ligands increases the levels of *CGB* transcript, hCG and β-hCG (106). PPARγ/RXRα heterodimers directly bind to the regulatory region of *CGB5* (106). Activation of PPARγ enhances the transcript levels of α-hCG and β-hCG in villous trophoblasts, but reduces the level of hCG in invasive extravillous trophoblasts (107). Shalom-Barak *et al* (108) and Peng *et al* (109) suggested that PPARγ is distinctly expressed in various trophoblast subsets and during trophoblast stem cell differentiation. *p53* selectively induces the expression of CGB7; a p53-responsive element has been identified in the promoter of CGB7 (101). MTA3, a chromatin-remodeling protein, acts directly on the *CGB5* promoter and suppresses *CGB5* expression in trophoblasts (102). The deregulation of MTA3 has previously been associated with pre-eclampsia (102).

In addition to transcription factors, various epigenetic mechanisms (methylation) are involved in the regulation of *CGB* expression in the placenta and cancer cells (110,111). Allelic polymorphism for methylation sensitivity in the promoter of *CGB5* have been shown to be associated with a high risk of miscarriage (112). The deregulation of epigenetic mechanisms with altered *CGB* expression has also been associated with an increased risk of early pregnancy loss (104).

9. Biological effects of β-hCG upregulation in human cancer

Proliferation. To explore the biological effects of trophoblastic differentiation in human cancer, *in vitro* experiments have been performed using several human cancer models. Ectopic expression of β-hCG appears to enhance the *in vitro* proliferation of ovarian (54) and stomach (34) cancer cells, but not that of CRC cells (20,35). Similar effects were noted on the *in vivo* proliferation of primary ovarian cancer cells (54), but not that of CRC (20,35) or bladder (113) cancer cells. Table I summarizes the various biological effects of β-hCG upregulation in human cancer.

Apoptosis. Overexpression of β-hCG inhibits the *in vitro* apoptosis of ovarian and bladder cancer cells (113), as well as that of cells obtained from the xenografts of nude mice (54). *In vitro* β-hCG treatment of bladder UC exerts antiapoptotic effects (113).

Migration. β-hCG reportedly stimulates the *in vitro* migration of *BRCA1*-mutant breast cancer (52), CRC (20,35), glioblastoma (114), ovarian cancer (19) and prostate cancer cells (115,116).

Invasion. β-hCG promotes *in vitro* cell invasion in *BRCA1*-mutant breast cancer (52), CRC (20,35), ovarian cancer (19), prostate cancer (115,116) and glioblastoma (114). β-hCG expression has also been strongly associated with tumor invasion in primary CRC (20,35), non-small cell lung cancer (36,37) and bladder cancer (4,117).

EMT. Ectopic expression of β-hCG in cancer cells appears to induce *in vitro* EMT in *BRCA1*-mutant breast cancer (52), CRC (20) and ovarian cancer (19). β-hCG has been reported to be essential for *in vivo* modulation of EMT in mouse tumor

Table I. Biological effects of β-hCG overexpression in human cancer.

First author, year	Cancer	Experimental model	Materials	Activated pathway	Poor prognosis	High expression	Invasion	Migration	Proliferation	Metastasis	EMT	Morphology change	Tumorigenesis	Anti-apoptosis	Chemoresistance	Angiogenesis	Recurrence marker	(Refs.)
Sengodan, 2017	Breast cancer	*In vitro*	MCF7 cells, MDAMB-231 cells, SKBR3 cells, T47D cells	TGFβRII signaling	N/A	V	V	V	N/A	N/A	V	N/A	V	N/A	N/A	N/A	N/A	(52)
Sengodan, 2017		*In vivo*	BRCA1 conditional knockout mouse model	Mutated/ defective BRCA1 activates β-hCG expression	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(52)
Kawamata, 2018	Colorectal carcinoma	*In vitro*	Caco-2 cells, LoVo cells, HCA7 cells, WiDr cells, T84 cells	EMT via TGF-β signaling pathway	N/A	N/A	V	V	X	N/A	V	V	N/A	N/A	N/A	N/A	N/A	(20)
Li, 2018			HCT-116 cells, HT-29 cells, SW480 cells	N/A	N/A	N/A	V	V	X	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(35)
Lundin, 2001		*In vivo*	Primary tumor	N/A	V	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(40)
Kawamata, 2018			Primary tumor	EMT via TGFN/ Aβ signaling pathway	V	V	V	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(20)

Table I. Continued.

First author, year	Cancer	Experimental model	Materials	Activated pathway	Poor prognosis	High expression	Invasion	Migration	Proliferation	Metastasis	EMT	Morphology change	Tumorigenesis	Anti-apoptosis	Chemoresistance	Angiogenesis	Recurrence marker	(Refs.)
Kawamata, 2018			LoVo-GFP cells, LoVo-hCGβ cells	EMT via TGF-β signaling pathway	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	V	N/A	N/A	V	N/A	(20)
Li, 2018			Primary tumor	N/A	V	N/A	V	V	X	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(35)
Konishi, 2018			Primary tumor	N/A	V	N/A	N/A	N/A	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	(38)
Białas, 2020	Esophageal carcinoma	*In vivo*	Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(118)
Li, 2013	Glioblastoma	*In vitro*	U87MG cells	ERK1/2 pathway	N/A	N/A	V	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	V	(114)
Białas, 2020	Head and neck cancer	*In vivo*	Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(118)
Li, 2013	Lung cancer	*In vivo*	Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(114)
Noda, 1990	Non-small cell lung cancer	*In vivo*	Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(39)
Arano, 1994			Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(41)
Okutur, 2010			Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(42)
Seder, 2017			Preoperative serum	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	V	(43)

Table I. Continued.

First author, year	Cancer	Experimental model	Materials	Activated pathway	Poor prognosis	High expression	Invasion	Migration	Proliferation	Metastasis	EMT	Morphology change	Tumorigenesis	Anti-apoptosis	Chemoresistance	Angiogenesis	Recurrence marker	(Refs.)
Wong, 2015			Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(44)
Khobta, 2012			Primary tumor, preoperative plasma	N/A	N/A	V	V	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(36)
Khattri, 2011			Primary tumor, preoperative serum	N/A	N/A	V	V	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(37)
Szturmowicz, 1999			Primary tumor, preoperative serum	N/A	V	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(16)
Vicier, 2013			Preoperative serum	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(45)
Dinis de Sousa, 2021			Primary tumor, preoperative serum	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(46)
Liu, 2017	Ovarian cancer	*In vitro*	ES-2 cells, SKOV3 cells	N/A	N/A	N/A	V	V	N/A	N/A	V	V	N/A	N/A	N/A	N/A	N/A	(19)
Sengodan, 2017			OVCAR8 cells	BRCA1 regulation on β-hCG	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(52)
Guo, 2011			T29 cells, T29-hCG cells, T80 cells, T80-hCG cells	N/A	N/A	N/A	N/A	N/A	N/A	V	N/A	N/A	N/A	N/A	V	N/A	N/A	N/A	(54)

Table I. Continued.

First author, year	Cancer	Experimental model	Materials	Activated pathway	Poor prognosis	High expression	Invasion	Migration	Proliferation	Metastasis	EMT	Morphology change	Tumorigenesis	Anti-apoptosis	Chemoresistance	Angiogenesis	Recurrence marker	(Refs.)
Śliwa, 2019		*In vivo*	Primary tumor	Demethylation regulating gene expression	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(103)
Liu, 2017			Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	V	V	N/A	N/A	N/A	N/A	N/A	(19)
Liu, 2017			ES-2 cells, SKOV3 cells	N/A	N/A	N/A	N/A	N/A	N/A	V	N/A	V	V	N/A	N/A	N/A	N/A	(19)
Guo, 2011			T29 cells, T29-hCG, T80 cells and T80-hCG cells were injected into an athymic nude mouse model	N/A	N/A	N/A	N/A	N/A	V	N/A	N/A	N/A	V	V	N/A	N/A	N/A	(54)
Guo, 2011			Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(54)
Sengodan, 2017	Prostate cancer	*In vitro*	DU145 cells	BRCA1 regulation on β-hCG	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(52)
Wu. 2006			DU145 cells, PC3 cells	N/A	N/A	N/A	V	V	N/A	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	(115)
Li, 2013			DU145 cells	ERK1/2 pathway	N/A	N/A	V	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(116)
Szturmowicz, 1995	Small cell lung cancer	*In vivo*	Preoperative serum	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	V	N/A	N/A	(138)

Table I. Continued.

First author, year	Cancer	Experimental model	Materials	Activated pathway	Poor prognosis	High expression	Invasion	Migration	Proliferation	Metastasis	EMT	Morphology change	Tumorigenesis	Anti-apoptosis	Chemoresistance	Angiogenesis	Recurrence marker	(Refs.)
Butler, 2000	Urothelial carcinoma	*In vitro*	T24 cells, SCaBER cells, J82 cells, 5637 cells, RT112 cells	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	V	V	N/A	N/A	N/A	(113)
Białas, 2020		*In vivo*	Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(118)
Cheng, 2021			Primary tumor	N/A	V	V	V	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	V	(4)
Hoshi, 2018			Postoperative serum	N/A	V	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	V	N/A	N/A	(10)
Rajabi, 2013			Preoperative serum, urine	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(53)
Armah, 2007			Primary tumor	N/A	V	V	N/A	N/A	N/A	V	N/A	N/A	N/A	N/A	V	N/A	N/A	(120)
Shimada, 2006			Primary tumor, preoperative serum	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(14)
Regalado, 2004			Primary tumor	N/A	N/A	V	V	N/A	N/A	V	N/A	N/A	N/A	N/A	V	N/A	N/A	(117)
Ramakumar, 1998			Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(47)
Oyasu, 1994			Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(139)
Białas, 2020	Uterine corpus endometrial carcinoma	*In vivo*	Primary tumor	N/A	N/A	V	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(118)

N/A, not available; V, reported in the literature; X, no significant differences; β-hCG, β-human chorionic gonadotropin; EMT, epithelial-mesenchymal transition.

xenograft models of CRC (20), CRC metastasis (20,38), large cell carcinoma (39) and lung squamous cell carcinoma (118).

Angiogenesis. Xenografts of CRC with aberrant β-hCG expression have been demonstrated to harbor a higher density of microvessels than control tumors in mice (20). The fifth subunit of β-hCG, CGB5, may also promote tumor growth and vasculogenic mimicry by activating the LH receptor signal transduction pathway (119).

Metastasis. Overexpression of β-hCG is strongly associated with increased metastatic potential in CRC (20,35), non-small cell lung cancer (36,37), ovarian cancer (19) and bladder cancer (4,117,120).

Tumorigenesis. Overexpression of β-hCG has been reported to enhance tumorigenesis in mouse tumor xenograft models of *BRCA1*-mutant breast cancer (52), CRC (20), ovarian cancer (19,54) and bladder cancer (113).

Taken together, these findings indicated that the molecular mechanisms underlying the effects of β-hCG on increased tumorigenesis include promotion of cell proliferation, differentiation, vasculogenesis, EMT and metastasis.

10. The hCG-mediated signaling pathways

TGF-β signaling pathway. In an early study conducted using CRC as a model, LoVo and HCA-7 cells were transfected with β-hCG (20). Ectopic expression of β-hCG was shown to upregulate zinc finger protein SNAI1 (SNAIL), zinc finger protein SNAI2, twist-related protein 1 (TWIST) and phosphorylated-SMAD2, but to downregulate epithelial cadherin in hCGβ-transfected HCA-7 cells compared with in control cells. The phosphorylation of SMAD2, which was activated through stimulation of TGF-β receptors during EMT, was also promoted. Together, these results indicated that β-hCG may induce EMT through the TGF-β signaling pathway. Fig. 1 depicts the β-hCG-mediated signaling pathways discussed in the present review.

EMT-related signaling pathway. A recent cell line and mouse model experiment revealed that β-hCG promotes the proliferation of gastric cancer cells *in vitro* through activation of the c-Met (121).

ERK1/2 signaling pathway. In human glioblastoma, β-hCG has been shown to upregulate the expression of phosphorylated-ERK1/2 in U87MG cells in a dose- and time-dependent manner (114). In addition, the ERK/matrix metalloproteinase 2 (MMP2) signaling pathway is involved in the β-hCG-mediated metastasis of epithelial ovarian cancer cells (122). This observation was supported by an *in vitro* experiment performed using the DU145 prostate cancer cell line (116).

11. Expression of non-hCG biomarkers associated with trophoblastic differentiation

In addition to hCG, the other trophoblastic markers that are used for diagnostic purposes include hPL, melanoma cell adhesion molecule (MCAM; also called CD146), p63, mucin (MUC)-4, human leukocyte antigen (HLA)-G, cytokeratin 18, inhibin A, GATA-binding protein 3 (GATA3), HSD3B1 and spalt-like transcription factor 4 (SALL4) (123-128).

The expression of these markers varies across trophoblast types, namely, cytotrophoblasts, syncytiotrophoblasts and intermediate trophoblasts (ITs); the latter two types may be derived from cytotrophoblasts. ITs may be further subtyped into villous ITs (villi are anchored to the basal plate through trophoblastic columns), implantation site ITs and chorionic-type (*chorion laeve*) ITs (129). Each subtype of IT has a different immunohistochemical profile and malignant counterparts. For example, hCG, HSD3B1, hPL, inhibin, MCAM, SALL4, HLA-G, MUC4 and p63 are expressed in the malignant villous ITs; hPL, MUC-4, HSD3B1, HLA-G and CD146 are expressed in the malignant implantation site ITs, with limited expression of hCG and inhibin; while tumor cells of chorionic-type IT origin diffusely express HSD3B1, HLA-G, p63, cyclin E, inhibin A and GATA3, with occasional expression of CD146 and hPL (130).

In general, SALL4 is specifically expressed in mononuclear cytotrophoblasts, in contrast to hCG, which is expressed mainly in syncytiotrophoblasts. HSD3B1 is regarded as a pantrophoblastic marker. HLA-G is useful for all three subtypes of IT. CD146 and hPL are highly specific for implantation site ITs, whereas p63 and PLAP are highly specific for chorionic ITs (129,131).

Notably, some of the aforementioned markers have been detected in nontrophoblastic tumors. For example, one small study (n=16) revealed that hCG was expressed in 93% of patients with UCTD (8). In this study, not only the trophoblastic component but also the UC component expressed hCG, at a rate as high as 85% (8); HSD3B1 was also expressed in the trophoblastic component of all but one case (8). By contrast, SALL4 expression was variable, with a 50% staining rate in trophoblasts and a 43% staining rate in the UC component of hCG-positive cases (8).

Although supplementary potential markers for trophoblastic differentiation have been identified, the application of some of these markers remains debatable, partly because of the difficulty associated with determining the trophoblastic lineage of candidate cells (132). The proposed characteristics of primary first-trimester trophoblasts include the expression of a specific set of protein markers (cytokeratin 7, GATA3 and TFAP2-γ), the HLA class I expression profile, the methylation of ELF5 and the expression of microRNAs (miRNAs) from the chromosome 19 miRNA cluster (132).

12. Options for targeted therapy

As a target for cancer vaccines, hCG has been explored for decades (133). An early investigation revealed the potential of a monoclonal antibody against β-hCG (6H1) in the inhibition of tumor growth *in vitro* and *in vivo* (134). The CDX-1307 vaccine (also called B11-hCG-β) was developed to target β-hCG-expressing bladder cancer cells (135). This vaccine comprises a B11 monoclonal antibody against the mannose receptor of antigen-presenting cells fused to β-hCG. In a phase I clinical trial involving patients with cancer, CDX-1307 was found to be well tolerated. It induced substantial β-hCG-specific cellular and humoral immune responses

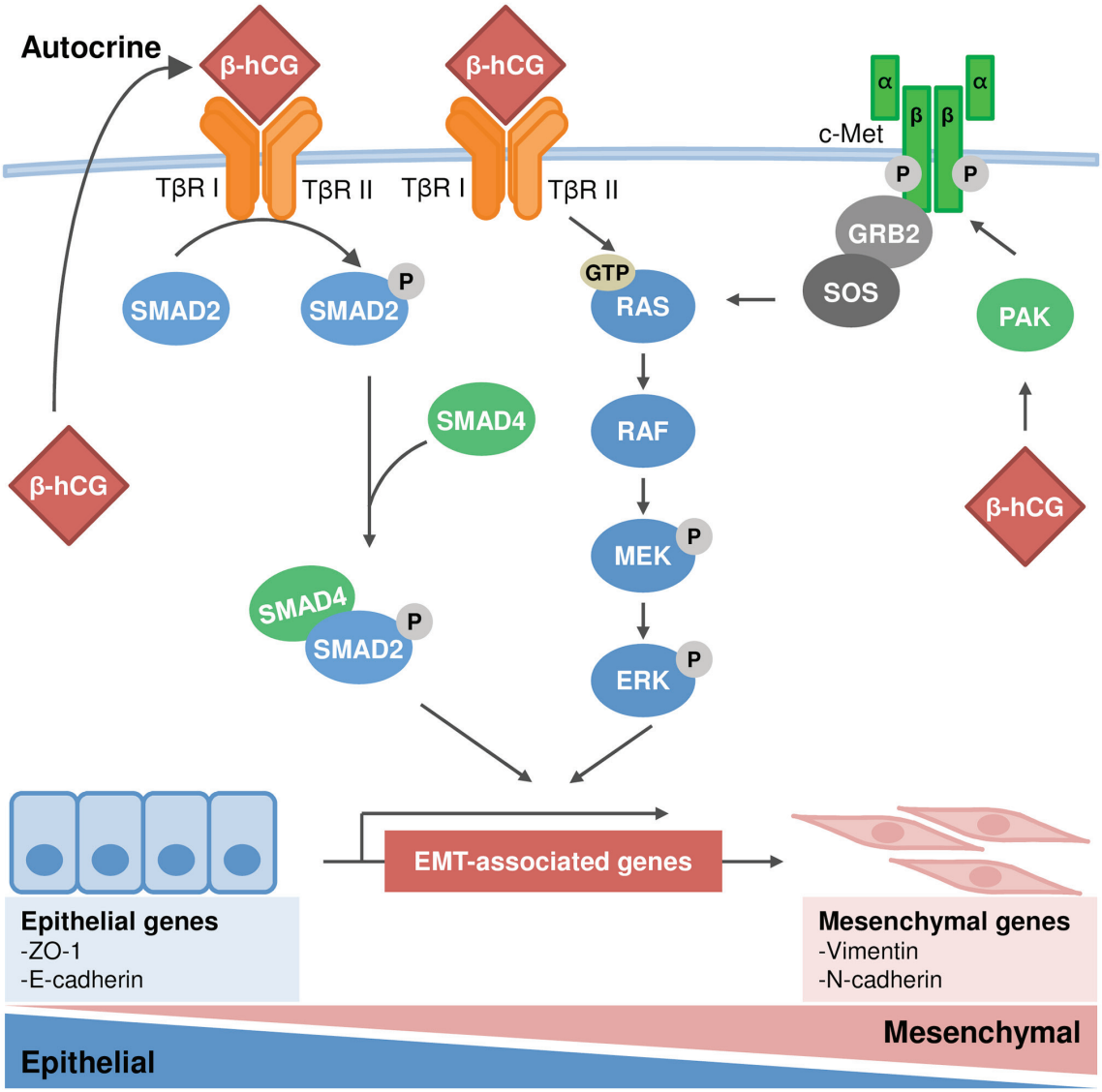

Figure 1. Schematic diagram of β-hCG-mediated signaling pathways in human cancer. To the best of our knowledge, the molecular mechanisms underlying β-hCG-mediated tumor progression have not yet been elucidated. The literature suggests that overexpression of β-hCG is involved in activation of the TGF-β/SMAD, MAPK/ERK and c-Met signaling pathways, resulting in the EMT of cancer cells in humans. β-hCG binds to the receptor for TGF-β, leading to activation of the SMAD and MAPK/ERK pathways. In addition, β-hCG upregulates c-Met through PAK signaling to activate the MAPK/ERK pathway. Ectopic expression of β-hCG induces EMT in carcinoma cells with upregulation of vimentin and the downregulation of E-cadherin. β-hCG, β-human chorionic gonadotropin; TβR I, transmembrane serine/threonine kinase type II receptor; TβR II, transmembrane serine/threonine kinase type I receptor; P, phosphorylated; EMT, epithelial-mesenchymal transition.

when co-administered with GM-CSF and the Toll-like receptor agonists Resiquimod (R848) and poly-ICLC (135). However, enrollment for the early phase II trial was slow and the study was terminated prematurely (https://clinicaltrials.gov/ct2/show/record/NCT01094496) (136).

Combination therapies can be used as alternatives. Through its direct and collaborative effects with Toll-like receptor ligands and accessory cell-secreted cytokines, hCG was shown to mediate chemoresistance in gonadotropin-sensitive tumors in a mouse study (137). The coadministration of curcumin and an anti-hCG vaccine (β-hCG conjugated to tetanus toxoid) to mice carrying syngeneic tumors resulted in considerably improved animal survival (137).

On the basis of the aforementioned molecular alterations, inhibitors of type I and II TGF-β receptors appear to successfully reverse the biological effects and overexpression of SNAIL and TWIST induced by β-hCG in human CRC (20). Moreover, an ERK1/2 inhibitor could reduce the expression of MMP2, invasion of human glioblastoma cells (114) and motility of prostate cancer cells *in vitro* (116). Taken together, these results indicated that both ERK1/2 and MMP2 are potential targets in precision therapy for β-hCG-related cancer progression.

13. Future perspectives

The understanding of the molecular basis of β-hCG-mediated tumorigenesis in human cancer remains incomplete. Information regarding the mechanisms underlying trophoblastic differentiation in human cancer may facilitate the development of personalized therapy for patients with cancer. With the advancement of research, targeting the constituent(s)

of β-hCG-mediated EMT and angiogenesis may improve current therapeutic regimens for patients with epithelial cancer with trophoblastic differentiation.

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Authors' contributions

CC, YLC, YWW, TL, HWC, CWH. KCL, YCO, CAC, CLH, CTL and NHC performed literature research. CC, YLC, CTL and NHC wrote the original draft. CC, CTL, WLC and NHC revised the article. YLC generated the original figure and table. CTL and NHC performed visualization. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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