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NEW RESEARCH HORIZON ***Review***

Recent progress in luteinizing hormone/human chorionic gonadotrophin hormone research

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ABSTRACT: The role of luteinizing hormone (LH) and human chorionic gonadotrophin hormone (hCG) in the regulation of normal reproductive functions in males and females is quite well established. Besides the use of hCG in the development of diagnostic immunoassays, it has been successfully used in the induction of final follicular maturation and ovulation in the assisted reproductive technologies. The basic and clinical research on the nongonadal actions of LH/hCG in the recent years has extended the potential of using these hormones in several clinical indications. Hereby we will analyze the advances in the LH/hCG research (briefly emphasizing the nongonadal research), which has the potential for multiple novel therapies in reproductive and the other areas of medicine.

Key words: luteinizing hormone / human chorionic gonadotrophin / luteinizing/human chorionic gonadotrophin receptor / gonadal and nongonadal effects

Introduction

Hypothalamic gonadotrophin-releasing hormone stimulated the secretion of luteinizing hormone (LH) by the anterior pituitary. Human chorionic gonadotrophin (hCG) is secreted by the placenta (Pierce and Parsons, 1981). Both LH and hCG are heterodimeric glycoprotein hormones belonging to cystine-knot growth factor families possessing the properties of cytokines and chemokines (Lapthorn *et al*., 1994). hCG is structurally related to LH and both hormones bind to the same LH/choriogonadotrophin receptor (LHCGR). LHCGR belongs to a family of seven-*trans* membrane spanning G-protein coupled receptors (McFarland *et al*., 1989; Ascoli*et al*., 2002). hCG is more potent than LH due to its higher receptor binding affinity and a longer circulatory half-life (Rao, 1979). Chorionic gonadotrophin is produced in primates, equines and in man, whereas LH is present in all species. hCG appears in the circulation around the time of implantation, increases to reach its peak about ninth week of pregnancy and then drops down to about one-tenth of the peak levels and remains there until the end of pregnancy. hCG is believed to be essential for the maintenance of the pregnancy by stimulating progesterone production by the corpus luteum gravidarum, although this concept was challenged by the discoveries of the multiple actions of hCG in nongonadal tissues throughout pregnancy and during labor (Lei and Rao, 2001a, b). hCG has been shown to stimulate testosterone production of fetal Leydig cells (Huhtaniemi and Pelliniemi, 1992; Huhtaniemi *et al*., 1977; Apaja *et al*., 2005). This review will concentrate on the LH/hCG in reproductive biology and general medicine with particular emphasis on nongonadal research.

The majority of the studies on nongonadal LH/hCG receptors were done on human rather than on other species (Rao and Lei, 2007). Although the studies on nongonadal LH/hCG actions led to a greater potential for novel therapeutic uses of hCG/LH than from the studies on the gonadal actions of LH/hCG (for a review, see Rao and Lei, 2007); few studies have been done in transgenic murine models, where physiologic significances of nongonadal LHR expression in reproductive function is questioned (Ahtiainen *et al*., 2007; Pakarainen *et al*., 2005, 2007). We will enclose a brief discussion on some recent studies based on transgenic murine models either overexpressing or showing disrupted genes, including analysis of the pros and cons of LH/hCG actions in nongonadal tissues.

The LH/hCG research on reproductive biology issues

Classical expression of the LHCGR has been well established in the testicular Leydig and ovarian theca, granulosa and luteal cells where

LH has been shown to regulate steroid hormone synthesis and gametogenesis (Segaloff and Ascoli, 1993; Dufau, 1998). LHCGR has been found in many nongonadal tissues in human and rodents. For example, human and rat adrenal glands (Pabon *et al*., 1996a; Apaja *et al*. 2005), cervix (Lin *et al*., 2003), fetal tissues (Abdallah *et al*., 2004), mammary gland (Tao *et al*., 1997b), oviduct (Lei *et al*., 1993b; Han *et al*., 1996; Zhang *et al*., 2001b), placenta (Reshef *et al*., 1990), uterus (Reshef *et al*. 1990; Han *et al*., 1997; Zhang *et al*., 2001b), sperm (Eblen *et al*., 2001) and many others.

LH/hCG research in ovulation induction, assisted reproductive technologies, pregnancy and miscarriages

The research on the chemistry of LH/hCG has led to the development of diagnostic immunoassays, which have been extensively used in reproductive medicine, such as intrauterine or ectopic pregnancy detection and reproductive cancer diagnostics (Davies *et al*., 2003). hCG is also very widely used for ovulation induction, where it is assumed that the beneficial effects come from its ovarian actions (Kafy and Tulandi, 2007; Rao and Lei, 2007). The actions of LH/hCG has been studied quite extensively in the control of ovarian functions, which have led to the development of widely used LH/hCG therapy to stimulate final follicular and oocyte maturation and ovulation induction (Filicori *et al*., 2005; Kafy and Tulandi, 2007). There is still an ongoing discussion on the supplementation of recombinant LH to the recombinant FSH with daily doses during the second half of the follicular phase, where some studies showed increased effectiveness (Tesarik and Mendoza, 2002; De Placido *et al*., 2005), whereas other studies, on the contrary, showed no evidence of increasing effectiveness (Nyboeandersen *et al*., 2008) on the ongoing pregnancy rates in the general population. The similar debate also includes whether some subgroups of women (aged >35 years) might benefit from LH activity supplementation during ovarian stimulation (Alviggi *et al*., 2006). The use of hCG to correct the luteal phase defect is not very popular nowadays (Dawood, 1994). A recent cohort study has shown that a subgroup of patients with a high rate of oocyte immaturity during a cycle stimulated with only recombinant (rec) FSH, and addition of LH (hMG, human menopausal gonadotrophin) in subsequent cycle increased significantly the number of mature oocytes and better quality embryos were obtained compared with only FSH cycles (Huddleston *et al*., 2009). The results of this study are quite convincing as the study design is done in a matched-pair design, where each patient serves as her own control (Huddleston *et al*., 2009). Further studies with bigger groups with similar criteria are needed in order to establish the appropriate clinical relevance for LH supplementation, which could increase the conception rate in assisted reproductive technologies. It has been shown that increased endometrial thickness and implantation rates could be achieved in the patients receiving hCG along with the GnRH analogs with estrogen/progesterone-supplemented oocyte recipients (Fujimoto *et al*., 2002; Tesarik *et al*., 2003). Another approach that might improve the implantation rates is the *in vivo* direct application of hCG to endometrium, which provokes endometrial morphological changes and cytokines production (Filicori *et al*., 2005).

hCG has been shown to possess a number of tropic actions in the reproductive tract and fetoplacental unit and plays an immunosuppressive role at the maternal–fetal interface (Lei and Rao, 2001a, b; Rao, 2001). hCG also has a relaxing effect on the uterine arteries, which increases the utero-placental perfusion (Toth *et al*., 2001). A single i.m. injection of hCG has been shown to decrease spontaneous as well as habitual miscarriage, as compared with Mg^{2+} treatment alone, and no maternal or fetal side effects could be observed (Toth *et al*., 2001).

Prevention of prematurity with hCG

Human myometrium contains LHCGR (Reshef *et al*., 1990; Han *et al*., 1997) and there exists also ample evidence on their functional relevance to an inhibition of contractions (Slattery *et al*., 2001; Belmonte *et al*., 2005; Phillips *et al*., 2005). Thus, it was hypothesized that hCG could also be a hormone contributing to myometrial quiescence in order to maintain the pregnancy. This putative tocolytic action of hCG was shown in a murine model (Kurtzman *et al*., 1999; Kurtzman *et al*., 2001). This tocolytic action was further demonstrated in women with preterm labor, although it was less effective in infection induced preterm labor cases (Than *et al*., 2003). It is likely that advanced infections reduce the efficacy of the hCG action, simply because the adverse changes have progressed too far for hCG to reverse or stop them. We believe that further cohort studies with larger populations are required before hCG can be recommended for routine use in prematurity prevention.

Potential role of hCG in preventing HIV/AIDS infection

Babies born to HIV-positive mothers are generally infected during the intrapartum period, when they are exposed to virus in maternal body fluids (Matheson *et al*., 1995). This observation prompted the basis for delivering babies of HIV-positive mothers after antiviral treatments by Cesarean sections, as instructed by the American College of Obstetricians and Gynecologists. It has been shown that the hCG suppressed HIV replication, reverse transcriptase, gene transcription and protein synthesis and prevents viral transmission from virus-positive lymphocytes to virus negative lymphocytes (Polliotti *et al*., 2002). In a transgenic HIV murine model, it was shown that hCG, but not the steroids, prevented the rapid progression of disease and premature demise of homozygous pups (De *et al*., 2002). These initial but quite promising results suggested that hCG could serve as a supportive drug for the potential treatment of HIV/AIDS along with the main antiviral drugs. Further studies are needed in order to establish the putative preventive role of hCG in HIV/AIDS.

Potential use of LH/hCG in endometriosis and their involvement in endometrial carcinomas

Endometriosis is characterized by the presence of endometrial tissue outside the uterus. This condition causes painful periods, chronic pelvic pain, subfertility and a profound reduction in quality of life, especially during women's reproductive years (Huber *et al*., 2004). Until now only surgery has been proved to provide definitive cure. There is insufficient evidence for hormonal use to completely cure this disease (Giudice and Kao, 2004; Berkley *et al*., 2005). The potential

use of hCG in the treatment of endometriosis was based on the findings that its symptoms ameliorate and lesions regress during pregnancy and menopause (Huber *et al*., 2004), ectopic implants contain LHCGR (Lincoln *et al*., 1992; Huber *et al*., 2004), and their activation by gonadotrophins leads to an inhibition of implants (Huber *et al*., 2004). hCG treatment had been shown to lead to significant and clinically relevant reduction in pain intensity and to greatly improved quality of life in women with therapy-refractory endometriosis (Huber *et al*., 2004). The same group went on further to show that hCG treatment altered the gene expression profile of stromal cells obtained from endometriotic lesions (Huber *et al*., 2007). Most of the up-regulated genes encoded proteins turned out to be involved with cell adhesion, intercellular communication, extracellular matrix remodeling, apoptosis and inflammation (Huber *et al*., 2007). Further studies are needed to validate therapeutic benefit of hCG use in endometriosis.

LHCGR is up-regulated in the endometrial carcinoma, even in the precursor lesions as comparison with normal endometrium. This finding suggested a potential role of the LH/hCG in endometrial carcinoma (Lin *et al*., 1994; Konishi *et al*., 1997). LH has been shown to have anti-apoptotic actions in human endometrial carcinoma cell lines, and post-menopausal obese women with endometrial carcinoma showed elevated levels of serum LH compared with age-matched obese women without the disease (Nagamani *et al*., 1992; Davies *et al*., 2000; Dabizzi *et al*., 2003). It would be important to know the molecular mechanisms underlying the up-regulation of LHCGR expression in endometrial carcinoma, which could suggest a new modality of treatment for this disease.

The LH/hCG research on some overall medical issues

LH/hCG in alzheimer disease research

Epidemiological studies showed a significant reduction in neurodegenerative disease among prostate cancer patients treated with GnRH analogs, which suggested a potential role for LH in alzheimer disease (AD) (Casadesus *et al*., 2005; Barron *et al*., 2006). LH is known to cross the blood–brain barrier and a very high number of LHCGR are mostly concentrated in the hippocampus, the region most vulnerable to AD) (Lei *et al*., 1993a). LH levels are also significantly elevated in both the serum and the pyramidal neurons of AD subjects compared with age-matched control subjects, which significantly correlated with the α-amyloid protein processing (Bowen *et al*., 2004; Casadesus *et al*., 2005; Barron *et al*., 2007). Genetically altered mice with exaggerated LH signaling showed behavioral changes that are consistent with the role of LH in promoting AD (Bowen *et al*., 2004; Casadesus *et al*., 2005; Barron *et al*., 2007). These findings suggest that it is not necessarily the estrogen deficiency alone, but rather chronically elevated LH levels with the dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis at menopause is a physiologically relevant signal that could promote neurodegeneration and predispose some post-menopausal women to develop AD (Bowen *et al*., 2004; Casadesus *et al*., 2005; Barron *et al*., 2007). AD features such as cognitive loss and amyloid beta deposition could be diminished by GnRH analog treatment, where LH signaling seems to be a useful therapeutic strategy. Clinical trials are underway for the treatment of AD using GnRH analogs (Atwood *et al*., 2005; Meethal *et al*., 2005) (also M.A. Smith, personal communication), which should provide further insights into the LH connection in AD.

Preventive actions of hCG for breast cancer

Completing a full-term pregnancy before the 20 years of age has a protective effect against breast cancer development in later life (Lei and Rao, 2000) which has been attributed to differences in the degree of differentiation in the breast (Russo *et al*., 1992). This protection effect appears to be due to the anticancer actions of hCG inducing differentiation of proliferative type to secretory type breast epithelial cells (Lei and Rao, 2000). The relevance of this protective effect has been extensively assessed in a rat model (Russo *et al*., 1990a, c, d; Russo and Russo, 1993), It showed that hCG protects the mammary gland against carcinogenic initiation and progression, mimicking the physiological process of pregnancy (Russo *et al*., 1990b, c; Russo and Russo 1993; Lei and Rao, 2000). In a recent study, hCG has been shown to induce apoptosis in breast cancer cells which may have a great potential to facilitate chemotherapeutic intervention and improve patient outcomes (Lopez *et al*., 2008). In this study, direct intratumoral injection of hCG into human breast cancer xenografts grown in nude mice increased the apoptotic index (Lopez *et al*., 2008). These results were supported by the findings that exposure to purified hCG decreased cell viability in five different breast cancer cell lines (Lopez *et al*., 2008). In some of these cell lines, the effects of hCG in cell viability appear to correlate with activation/expression of the hCG/LH receptor (Lopez *et al*., 2008). The authors suggested that preoperative apoptotic induction by hCG may improve local control or work synergistically with neoadjuvant chemotherapy to improve complete pathologic response of locally advanced breast cancer (Lopez *et al*., 2008). In light of these above-mentioned studies, hCG may have potential as a preventive measure against carcinogenic initiation and progression as well as its pro-apoptotic action opening the possibility for hCG to facilitate chemotherapeutic initiatives.

Transgenic murine models on nongonadal actions of LH/hCG

The presence and localization of LHCGR in the reproductive tract of wild-type mice has been analyzed (Zhang *et al*., 2001b). LHCGR mRNA expression has been shown in stromal cells of the wild-type murine endometrium and in the uterine serosa (Zhang *et al*., 2001b). Uterine smooth muscle cells had low levels of expression, and the endometrial epithelium was negative, whereas in the oviduct, high levels of LHCGR expression were noted on the serosa and in subepithelial cells (Zhang *et al*., 2001b). Oviduct smooth muscle had low expression, and the epithelium was negative (Zhang *et al*., 2001b). The nongonadal LHCGR have been suggested to be physiologically redundant on the basis of a LHCGR disrupted transgenic murine model, LuRKO, and said to come into play when pharmacological doses of hormones are administrated (Pakarainen *et al*., 2005). This speculation is not correct, as receptors in the nongonadal tissues, similarly in the gonads, have been activated by similar hormone concentrations (Kananen *et al*., 1997; Kiiveri *et al*., 1999; Kero *et al*., 2000; Rahman *et al*., 2004). Two independent groups reported two different LHCGR knock out murine models (LHRKO and LuRKO), with clear and rather similar phenotypes

with completely eliminated functional LHR in the (−/−) mice (Lei *et al*., 2001; Zhang *et al*., 2001a). The LHRKO model was created by targeted deletion of the proximal part of the *LHR* promoter and exon 1 (Lei *et al*., 2001), and the LuRKO model by targeted disruption of the long 11th exon of LHR, encoding the transmembrane and intracellular domains of the receptor (Zhang *et al*., 2001a). Discrepancies on phenotypic interpretation between these models occur regarding the evidence for or against the functional significance of nongonadal LHCGR action (Chudgar *et al*., 2005; Pakarainen *et al*., 2005). LHR null mice with transplanted wild-type ovaries in LuRKO (Pakarainen *et al*., 2005) mice could become pregnant, but not in LHRKO (Chudgar *et al*., 2005) mice. The pregnancy failure in the latter case was predictable because of the uterine genes involved in implantation are dependent on the uterine LH/hCG actions. It is highly likely that the strategies used in receptor silencing could be the reason for this discrepancy (Lei *et al*., 2001; Zhang *et al*., 2001a). Actually LuRKO mice has been used successfully in another uterine study in order to prove the functionality of the uterine LHCGR, where in mice aortic ring study, angiostimulation by recombinant hCG was abrogated completely by deletion of LHCGR, i.e. as in LuRKO mice (Berndt *et al*., 2006). This study additionally showed the angiogenic activity of hCG through LHCGR on endothelial epithelial cells of the endometrium (Berndt *et al*., 2006).

Murine transgenic (TG) models have been very productive in demonstrating the nongonadal adrenocortical LHCGR functionality. LHR expression in the murine adrenal gland is an exception and not found in wild-type (WT) animal (Kero *et al*., 2000; Rahman *et al*., 2004). Prepubertally gonadectomized inhibin null mice *(inh−/−)* (Matzuk *et al*., 1992; Matzuk *et al*., 1994) and transgenic mice under the inhα promoter fused with SV40 T antigen oncogene (inhα/Tag) express adrenocortical LHCGR and have a distinct adrenal phenotype emphasizing the nongonadal LHCGR effects. Gonadectomized *inh−/−* and inhα/Tag develop adrenocortical tumors in 100% penetrance, demonstrating that inhibin is also a tumor suppressor for the adrenal gland. The appearance and growth of adrenal tumors in inhα/Tag mice were found to be gonadotrophin dependent, since they failed to appear after functional gonadectomy induced either by administration of a GnRH antagonist or by cross-breeding the TG mice into the hypogonadotropic hpg genetic background (Cattanach *et al*., 1977; Kananen *et al*., 1997). The post-gonadectomy elevation of LH levels apparently induced the ectopic LHCGR expression in the adrenal cortex, which together with the potent oncogene Tag co-expression triggered adrenocortical tumorigenesis (Rahman *et al*., 2001, 2004). Inhα/Tag adrenocortical mice additionally have very successfully been used to test the hypothesis that adrenocortical tumors possessing LHCGR could be selectively destroyed by a lytic peptide hecate, conjugated to CGβ subunit (Vuorenoja *et al*., 2008; Vuorenoja *et al*., 2009). TG female mice-expressing LHβ-CTP (a chimeric protein derived from the β-subunit of bovine LH and a fragment of the β-subunit of hCG) exhibit elevated serum LH, infertility, polycystic ovarie, and ovarian tumors (Risma *et al*., 1995). Intact TG βLHβ-CTP females with enhanced ovarian estrogen synthesis have been shown to be involved in increased secretion of prolactin (PRL), which consequently elevated the LHR expression of female mice with chronically elevated LH (Kero *et al*., 2000). LuRKO 9- to 10-week-old female mice exhibited decreases in bone histomorphometric parameters tested, indicating that the loss of LH signaling results in a reduction in bone formation or an increase in bone resorption (Yarram *et al*., 2003). All these above-mentioned TG murine model reports strongly support the nongonadal significance of LH/hCG and LHCGR.

TG mice overexpressing hCG (hCGβ and common α-subunits under the human ubiquitin C promoter), producing 3–4-fold elevation in males, 30-fold in females (hCGα or hCGβ) or drastically 1000-fold elevated levels of circulating bioactive LH/hCG in hCGαβ mice, compared with WT-littermates (Rulli *et al*., 2002, 2003). Clear nongonadal phenotypes were also observed in these hCG overexpressing TG mice: the females developed obesity, pituitary macprolactinomas, mammary gland adenocarcinomas and elevated bone density in hCG*β*(Rulli *et al*., 2002, 2003; Yarram *et al*., 2003), or in hCGαβ+ mice: germ cell tumors in females and prostate hyperplasia, lower urinary tract obstruction and hydronephrosis and dilated urinary bladder in males (Rulli *et al*., 2003; Pakarainen *et al*., 2007). However, all the nongonadal phenotypes of hCGβ+ could be abolished or prevented after gonadectomy, indicating abnormal gonadal hormone production, rather than direct nongonadal hCG effects, could be responsible for the nongonadal phenotypes observed in hCGβ+ females or in hCGαβ+ mice (Rulli *et al*., 2002, 2003; Yarram *et al*., 2003; Pakarainen *et al*., 2007). No adrenal gland LHCGR expression has been reported in these hCG overexpressing mice. These examples do not support the nongonadal actions of LH/hCG and LHCGR, as they show even in the presence of very (30-fold) or extremely high (1000-fold) levels of hCG there were no nongonadal phenotypes in mice caused by nongonadal LHCGR. The overexpression models may not be highly useful in deciphering the information on the importance on nongonadal LH/hCG receptors. This is simply because unusually high hCG/LH levels could indeed bypass nongonadal targets as the receptors in them could be selectively down-regulated resulting in abrogation of response.

Analyzing the evidence for and against nongonadal effects of LH/hCG through LHCGR from transgenic mice research, we would argue that the evidence for their importance is much stronger than that against. For instance, the LuRKO mice getting pregnant after ovary transplants as explained above could be due to the different receptor silencing methodology used. With regard to the uterine issue, we believe that a uterine specific LHR knockout model should be developed in order to prove the functionality of uterine LHCGR. As for hCG overexpressing models, we believe that when the circulating bioactive LH/hCG, are so pathologically high (either 30-fold or more than 1000-fold) as compared with WT littermates, that they could drive the gonads excessively masking nongonadal actions. Moreover, very high hCG levels could be just as ineffective as very low levels because they may down-regulate the receptors more rapidly in nongonadal tissues than in gonadal tissues (Table I).

A novel therapeutic approach to treat endocrine tumors through their LHCGR by hecate chorionic gonadotrophin β conjugate lytic peptide

Improvements in cancer research are a big challenge of medical research. Despite the immense efforts made in the improvement of

Table I Murine transgenic models providing evidence for or against extragonadal actions of LH/hCG.

Genetically targeted overexpressing/ disrupted TG murine models (model name)	References	Evidence for/against the nongondal effects of LHCGR—organ specificity (ref)	Gonadectomy required in order to express the LHCGR
LHR knockout mice (LuRKO)	(Zhang *et al*., 2001a, b).	Against—uterus (Pakarainen *et al*., 2005) For—uterus (Berndt *et al*., 2006); bones (Yarram *et al*., 2003)	NO
LHR knockout mice (LHRKO)	(Lei *et al*., 2001)	For—uterus (Chudgar *et al*., 2005; Lin *et al*., 2005a, b)	NO
Inhibin null mice *(inh−/−)*	(Matzuk *et al*., 1992)	For—adrenals (Matzuk *et al*., 1994)	YES
Inhibin α promoter SV40 T antigen mice (inhα/Tag)	(Kananen *et al*., 1996)	For—adrenals (Rahman *et al*., 2001, 2004; Vuorenoja *et al*., 2008, 2009)	YES
Mice expressing a chimeric protein derived from the β-subunit of bovine LH and a fragment of the β-subunit of hCG (LHβ-CTP)	(Risma *et al*., 1995)	For—adrenals (Kero *et al*., 2000)	NO
Mice overexpressing *hCGβ* and *common α-subunits* under the human ubiquitin C promoter *(hCGβ+ and/or hCGαβ+)*	(Rulli *et al*., 2002, 2003)	Against—pituitary, mammary gland, bone, adrenals (Rulli *et al*., 2003, 2002; Yarram *et al*., 2003)	NO

diagnosis and treatment, cancer remains a major concern and cause of morbidity and mortality. Majority of the available anti-neoplastic therapies have severe side effects, where tumor cells often develop drug resistance. We have developed a receptor-based therapy (LHCGR), using lytic peptides, as they appear to selectively kill cancer cells due to change of their membrane potential, where most tumor cells possess a negatively charged outer layer which directs the action of lytic peptides towards the tumor cells and kills them, but spares the healthy ones even with LHCGR (Leuschner and Hansel, 2004). Hecate CGβ conjugate (Leuschner *et al*., 2001) is a fusion polypeptide of 23-amino acid hecate, an amphiphatic lytic peptide, synthetic analog of mellitin, the principal toxic component of natural honeybee venom, which was tethered with a 15 amino acid (81–95) fragment of hCGβ subunit responsible for the LHCGR binding (Morbeck *et al*., 1993). It has been further proven that the CGβ chain possesses high receptor affinity towards LH receptors and hecate CGβ conjugate selectively destroys cells expressing the LHCGR (Leuschner *et al*., 2001; Bodek *et al*., 2003; Bodek *et al*., 2005a, b). The cytotoxic activity of the conjugate induces plasma membrane disruption in a short period of time (Bodek *et al*., 2005b). The efficacy of hecate CGβ conjugate has been investigated with significantly successful results with no detectable side effects in prostate cancer (Hansel *et al*., 2001; Leuschner *et al*., 2001, 2003b; Bodek *et al*., 2005a), mammary tumors (Bodek *et al*., 2003; Leuschner *et al*., 2003a) as well as in ovarian cancer (Gawronska *et al*., 2002; Bodek *et al*., 2005b), testicular tumors (Bodek *et al*., 2005b), and finally adrenal tumors (Vuorenoja *et al*., 2008), all of which possesses LHCGR. Hecate-CGβ conjugate induced a rapid and cell-specific membrane permeabilization of LHCGR expressing cells *in vitro*, suggesting a necrotic mode of cell death, without activation of apoptosis (Bodek *et al*., 2005b). The necrotic mode of cell death was also apparent in prostate cancer cells (Bodek *et al*., 2005a). Clinical studies will be able to provide more evidence on their effectiveness and limitations on human endocrine cancers expressing LHCGR (Pabon *et al*., 1996b; Lojun *et al*., 1997; Meduri *et al*., 1997; Tao *et al*., 1997a).

Conclusions and future directions

hCG, as a therapeutic drug is rather nontoxic with negligible side effects, if any. It is an inexpensive drug as compared with other drugs used for any of the above-mentioned medical conditions and with the advancement of DNA recombinant technology, the scaling up of hCG production became much easier. This extremely low toxicity, easy availability and extensive research makes hCG an important choice for treating or for prevention of several diseases, as mentioned in this review. Lytic peptide hecate CGβ conjugate, which kills selectively the LHCGR possessing cells, sparing the healthy normal cells also opens up a new possibility perhaps beginning as a supplement for synergistic or additive treatment effects with existing chemotherapeutic agents or with other forms of cancer treatment, rather than replacing them. In this regard, the cytotoxicity against the healthy cells could be reduced and lytic peptide mediated destabilization of cancer cells and may even confer chemosensitivity on cancer cells with multi-drug resistance phenotype. The discovery that hCG/LH can act on nongonadal tissues represents a paradigm shift. Although it is obvious that a lot more intensive research is still needed, the current state of knowledge reaffirms that the physiological actions of hCG and LH include nongonadal targets along with the gonadal targets. The studies on nongonadal LH/hCG actions lead to a greater potential for novel therapeutic LH/hCG uses than all the previous studies on the gonadal actions of LH/hCG.

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