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Association of hCG and LHCGR expression patterns with clinicopathological parameters in ovarian cancer

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ABSTRACT

In addition to its critical role during pregnancy, human chorionic gonadotropin (hCG) has been shown to be expressed by various tumor types. Recent studies have similarly documented the presence of the luteinizing hormone (LH)/hCG receptor (LHCGR) in a variety of nongonadal organs; however, its clinicopathological significance in ovarian cancer remains unclear. The present study used a combination of immunohistochemical, real-time PCR, and western blot analyses to examine hCG and LHCGR expression in normal and cancerous tissues collected from patients with epithelial ovarian cancer (EOC). hCG and LHCGR expression levels were resultantly shown to be significantly increased and decreased in cancerous versus normal (or benign) ovarian tissues, respectively ($P < 0.05$), and both expression pattern changes were associated with more advanced tumor stages and a higher rate of metastasis. Furthermore, patients with tumors with high or low levels of hCG and LHCGR, respectively, experienced a worse overall survival (OS) rate than those with low hCG or high LHCGR expression levels ($P < 0.05$). In fact, hCG and LHCGR expression levels were independent prognostic factors of patient OS ($P < 0.05$) for EOC. Collectively, these findings indicate that hCG and LHCGR expression pattern changes are associated with EOC occurrence and progression. Thus, hCG and LHCGR represent promising potential targets to improve the diagnosis, treatment, and prognosis of patients with EOC.

1. Introduction

Epithelial ovarian cancer (EOC) is a gynecological malignancy that incurs sufficiently high mortality rates worldwide to be the most lethal gynecological cancer, and the fifth leading cause of cancer-related death in women [1]. For all cancer types, the clinical factors that determine patient prognosis include the state (i.e. the histological subtype, grade, and stage) of the disease at diagnosis, and the extent of residual disease after surgery [2]. EOC induces a high fatality rate largely due to the fact that its onset is often occult, causing it to be detected late. Once diagnosed, the standard treatment for patients with EOC comprises a combination of surgery and chemotherapy [3]. To improve the current prognosis for these patients, more sensitive markers and novel therapeutic targets are urgently needed to facilitate the early detection and effective treatment of ovarian cancer, respectively.

Human chorionic gonadotropin (hCG) is released by the syncytiotrophoblast of the placenta (which consists of an α and a β subunit), and exists as five independent molecules in the body [4]. In addition, hCG (particularly subunit β-hCG) is also expressed by normal non-trophoblastic tissues, predominantly including those of the testes, prostate, thymus, skeletal muscles, and pituitary glands [5]. Recent reports have shown that β-hCG is also secreted by a variety of malignant tumor types, including ovarian, cervical, vaginal, gastrointestinal, bladder, lung, colorectal, and prostate tumors [5–8]. In fact, hCG has been suggested to promote ovarian cancer progression and metastasis by inducing epithelial-mesenchymal transition (EMT) and inhibiting apoptosis [9,10]. The EMT transforms epithelial cells into cells with migration and invasion, and it is thought to be involved in the metastasis process of epithelial tumors [11].

The heptahelical luteinizing hormone/choriogonadotropin receptor (LHCGR) regulates ovulation by binding both the luteinizing hormone (LH), (which is a product of the anterior pituitary), and hCG. It has also been shown to be expressed by multiple cancer types; for example, LHCGR has been shown to be more highly expressed in human endometrial carcinomas than in normal endometrium, and its expression is associated with a poor prognosis in patients with endometrial cancer

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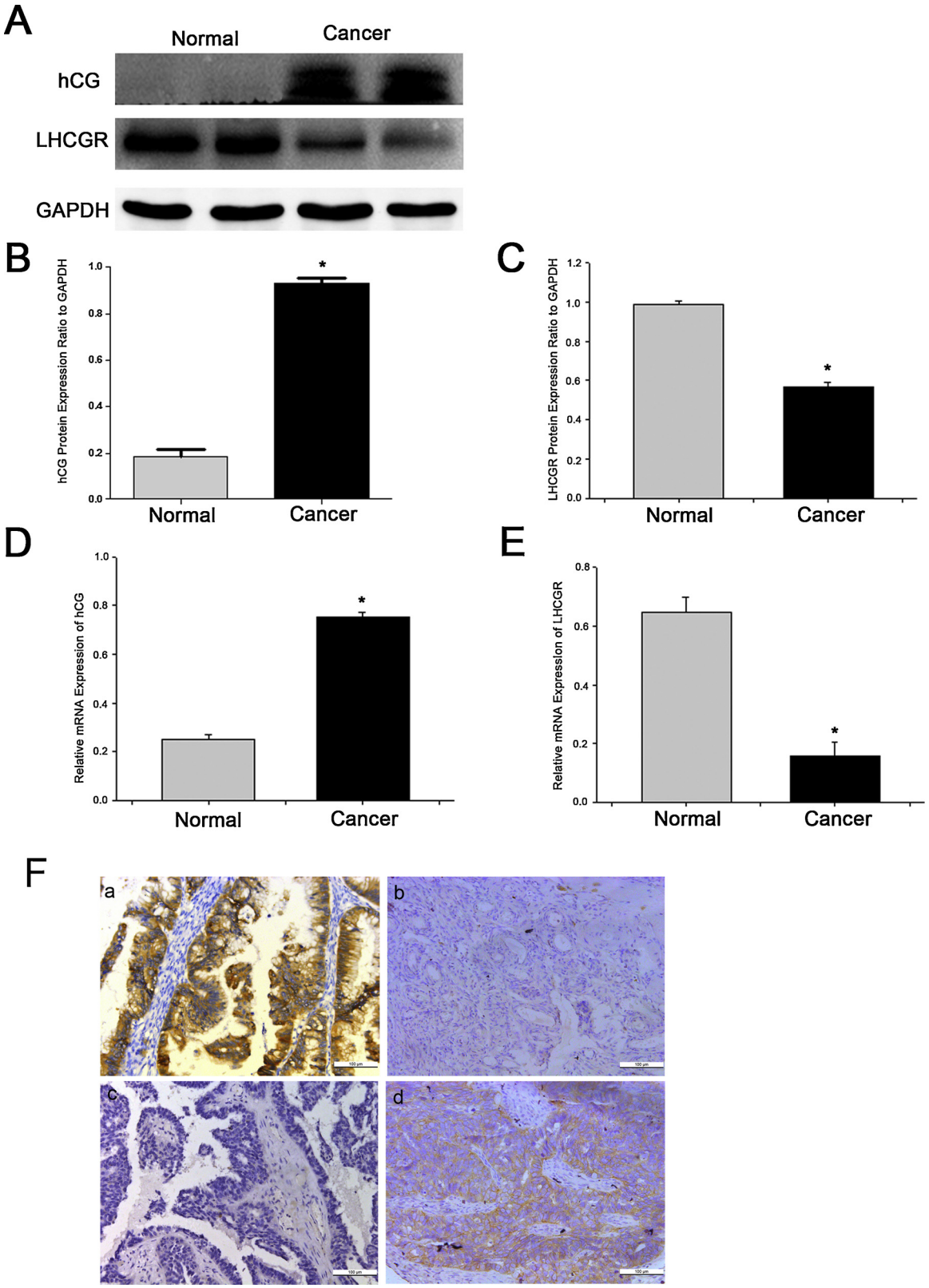

Fig. 1. hCG and LHCGR gene and protein expression in normal and cancerous fresh ovarian tissues collected from patients with epithelial ovarian cancer (EOC). (A) hCG and LHCGR protein expression in normal and cancerous ovarian tissues were compared via a western blot analysis. GAPDH was used as a loading control. (B) Bar chart showing the ratio of hCG to GAPDH protein production. hCG expression was significantly higher in cancerous than normal ovarian tissues. (C) Bar chart showing the ratio of LHCGR to GAPDH protein production. LHCGR expression was significantly lower in cancerous than normal ovarian tissues. (D, E) PCR analyses of (D) *hCG,* and (E) *LHCGR* mRNA expression in normal and cancerous ovarian tissues. 18S was used as a loading control for both analyses. All protein and mRNA levels were quantified via a combined analysis of three independent replicate experiments. *$P < 0.05$. (F) IHC staining for hCG (left) and LHCGR in ovarian cancer tissue. (a) strong staining of hCG in ovarian cancer tissue; (b) low staining of hCG in noncancerous ovarian tissue; (c) negative staining of LHCGR in ovarian cancer tissue; (d) strong staining of LHCGR in noncancerous ovarian tissue.

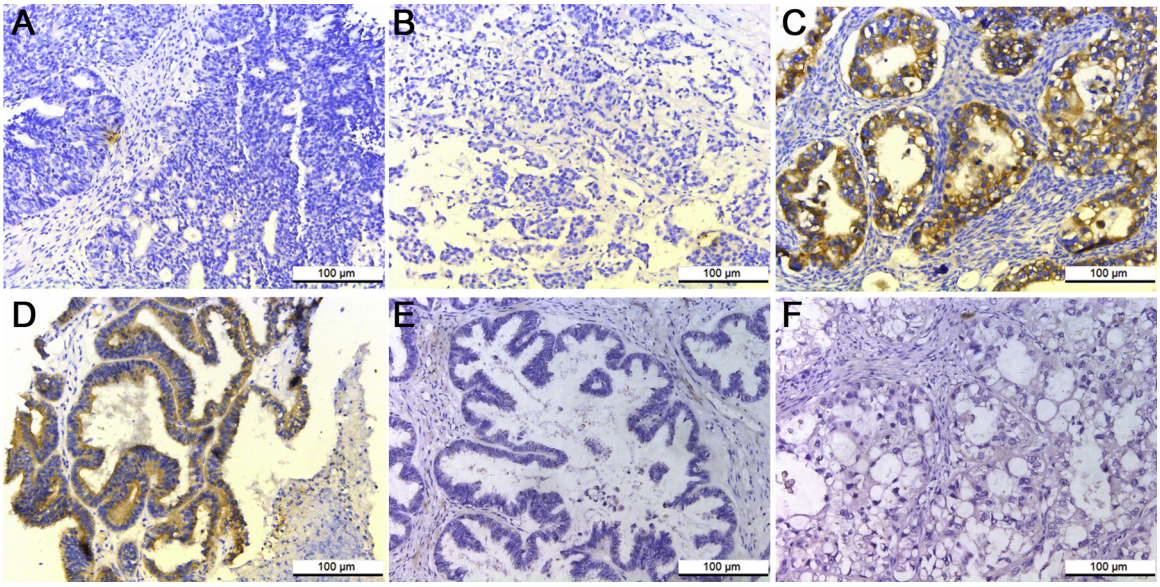

Fig. 2. Immunohistochemical (IHC) analysis of hCG and LHCGR expression in tissue microarrays (TMA) collected from patients with epithelial ovarian cancer (EOC). (A–C) IHC staining for hCG expression produced (A) no, (B) weak, and (C) strong staining in normal, benign, and malignant ovarian tissues, respectively. (D–F) IHC staining for LHCGR expression produced (D) strong, (E) weak, and (F) no staining in normal, benign, and cancerous ovarian tissues, respectively. Original magnification, ×200. Scale bar, 100 μm.

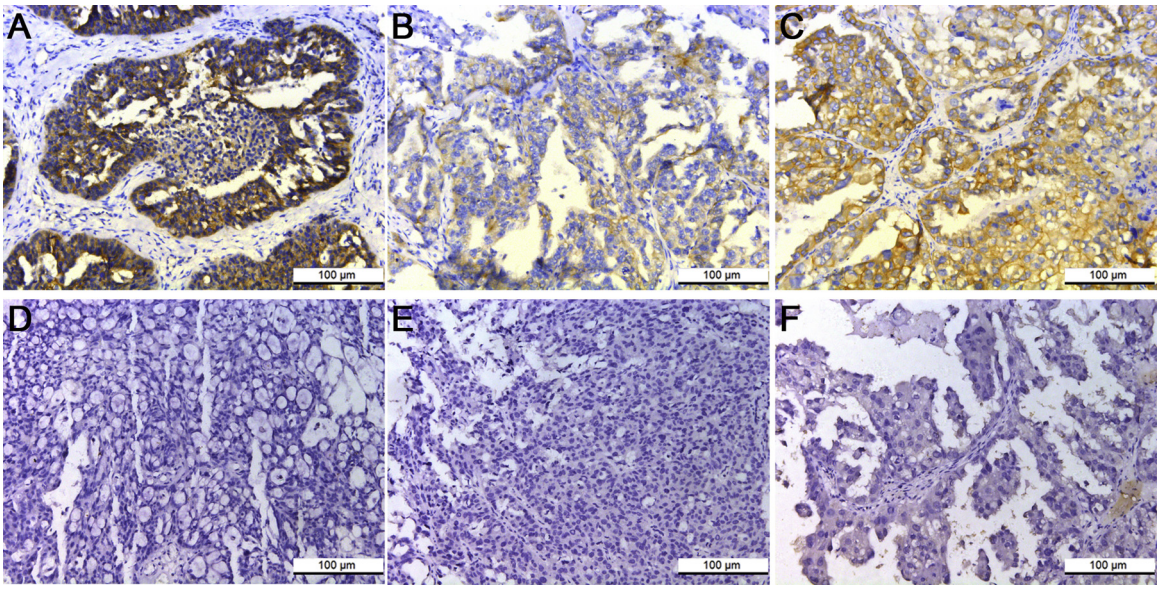

Fig. 3. hCG and LHCGR expression in different epithelial ovarian cancer (EOC) samples of varying histological classifications. (A–C) Positive immunohistochemical (IHC) staining of hCG in (A) serous, (B) endometrioid, and (C) clear-cell ovarian cancer. (D–F) Negative IHC staining of LHCGR in (D) serous, (E) endometrioid, and (F) clear-cell ovarian cancer. Original magnification ×200. Scale bar, 100 μm).

[12,13]. Similarly, Noci et al. [14] previously showed that LHCGR expression correlates with human primary endometrial cancer invasiveness in vitro, and LHCGR protein is produced by both normal and neoplastic endometrium. Moreover, the presence of LHCGR mRNA and protein has also been documented in breast cancer cell lines and endometrioid, adrenal, and prostate tumors [8,15].

Nevertheless, neither LHCGR expression in EOC, nor its correlation with patient clinical parameters, has yet been directly evaluated; thus, it is not clear whether hCG and/or LHCGR expression directly modulate tumor growth and/or disease progression in EOC [16–18]. Our research group previously showed that treating OVCAR-3 cells with different hCG concentrations in vitro does not significantly affect the expression of the hCG receptor [19]. In the present study, we performed quantitative polymerase chain reaction (qPCR) and western blot assays to analyze the mRNA and protein expression levels of hCG and LHCGR, respectively, in fresh ovarian epithelial cancer tissues, and in tissue arrays collected from patients with EOC. We furthermore evaluated whether changes of hCG and LHCGR expression patterns correlate with the clinical/pathological features (FIGO, grade, histological classification, ascites cell, serum CA125(U/ml) and metastasis), and/or the prognosis of patients with EOC. The Federation of Gynecology and Obstetrics (FIGO) stage system is widely used for grading endometrioid carcinomas which is based on architecture. Tumours with ≤50% solid glandular component are grade low, and tumours with > 50% of solid glandular component are classified as grade high. The FIGO, grade, histological classification and metastasis are associated the chemotherapy and therapy method, serum CA-125 levels may be a specific biomarker for EOC [20].

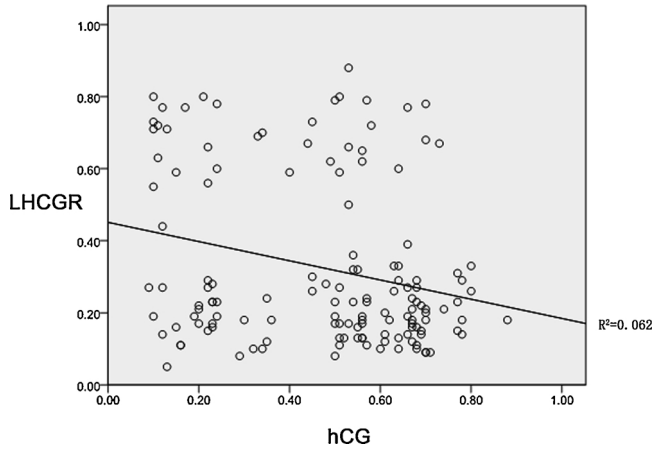

Fig. 4. Correlation between hCG and LHCGR expression in ovarian cancer. There was an obvious negative correlation between hCG and LHCGR expression levels in the analyzed ovarian cancer tissues (R = −0.249, P = 0.003).

2. Materials and methods

2.1. Clinical data and tissue samples

The present study enrolled 242 patients who were admitted to the Affiliated Hospital of Nantong University, China, between January 2005–December 2012. The median age of the patients was 55.05 years (range 24–80 years). Samples collected from the patients included 24 normal ovarian and 24 normal fallopian tube (control) samples, as well as 12 benign, 43 borderline, and 139 EOC ovarian tumor samples. The inclusion criteria were as follows: all patients provided complete clinical information to the study, were diagnosed by the pathologist, and had undergone no preoperative chemotherapy, radiotherapy, or immunotherapy. Tumor histological grades and clinical stages were determined pathologically after surgery. All fresh tissues samples (including 18 ovarian cancers and 5 noncancerous ovarian tissues: ovarian cyst and serous cystadenoma) for real-time (RT)-PCR and western blot assays were collected from the patient cohort at the Affiliated Hospital of Nantong University between March 2017–February 2018. The study protocol was approved by the Ethics Committee of the Affiliated Hospital of Nantong University, and all experiments were performed in line with approved guidelines of the Affiliated Hospital of Nantong University. All study patients provided written informed consent for their participation in the study.

2.2. Immunohistochemical (IHC) staining and evaluation

All IHC analyses were performed using the tissue microarray system (Quick-Ray, UT06, UNITMA, Korea) utilized by the Department of Clinical Pathology, Nantong University Hospital, Jiangsu, China. Tissue MicroArray (TMA) specimens were cut into 4-μm sections and placed on super frost-charged glass microscope slides. These were then twice incubated in a xylene liquid tank for 15 min, before being heated in a constant temperature oven at 60 °C for 6–8 h. The sections were then passed through an ethanol series comprising 100% (4 min), 95% ethanol (4 min), 80% (2 min), and 70% (2 min) ethanol (diluted in ddH2O, which is double distilled H_2O), before being rinsed in tap (2 min) and ddH_2O (2 min). They were then immersed in citrate buffer, and pressure-cooked (3 min) to facilitate antigen retrieval. After cooling to room temperature, the sections were blocked (20 min) with 3% H_2O_2, washed three times (5 min) in PBS, spin-dried, and incubated with primary hCG (ab187285, 1:100 dilution) and LHCGR (ab204950, 1:100 dilution) antibodies (4 °C, overnight). After a further three (5 min) PBS washes, the sections were incubated (30 min) with secondary antibody reagents. The three-step immunohistochemistry kit was bought by Beijing Zhongshan Jinqiao Company. Color was developed using a coloring solution, DAB (containg concentrate and diluents), concentrate: 1:20 dilution before the sections were counterstained with hematoxylin (10–20 s) and dehydrated in an ethanol series comprising 70% (3 min), 80% (3 min), and 95% ethanol (5 min), and anhydrous ethanol (5 min). Finally, the sections were made transparent with xylene, and sealed using neutral resin. All scores were assigned by two independent pathologists, without knowledge of patient clinical or pathological factors, using a Leica fluorescence microscope (Wetzlar, Germany). If the judgment results of the two pathologists were different, a third person evaluated again. hCG and LHCGR expression was visualized via immunohistochemical staining (SP method), the intensity of which was scored as either 0 (negative), 1 (weakly positive), 2 (moderately positive), or 3 (strongly positive). The percentage of

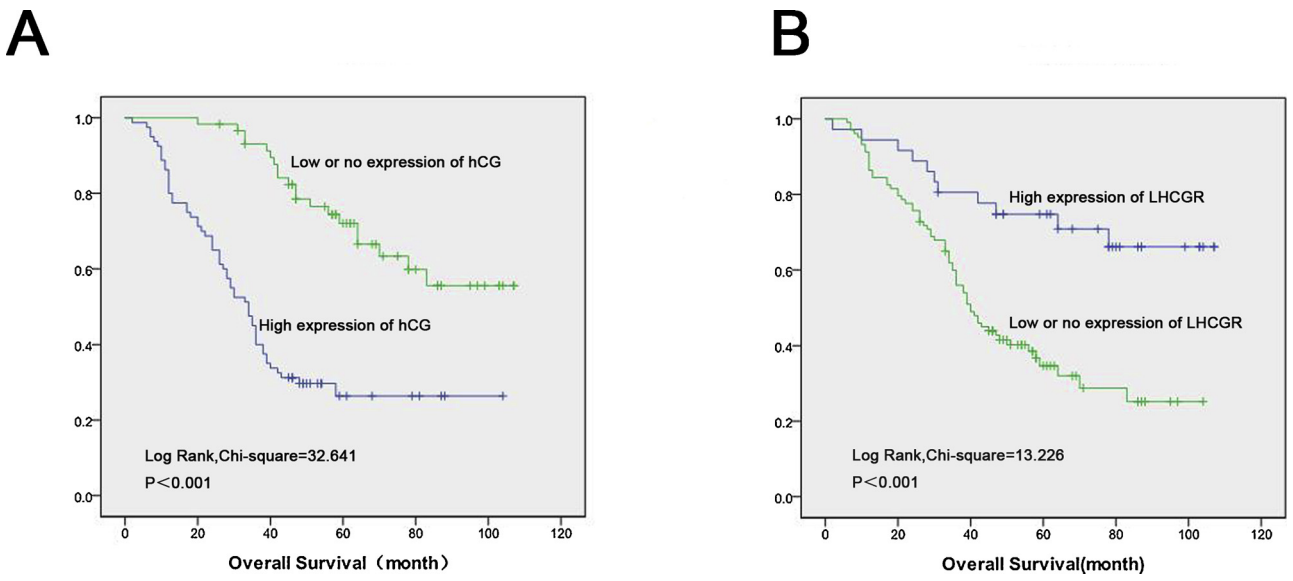

Fig. 5. Kaplan-Meier curves constructed using the log-rank survival test. (A) The overall survival (OS) rate experienced by patients that exhibited tumors with high hCG expression was significantly lower than that experienced by patients that exhibited tumors with low or no hCG expression (P < 0.001). (B) The OS rate experienced by patients that exhibited tumors with high LHCGR expression was significantly higher than that experienced by patients that exhibited tumors with low or no LHCGR expression (P < 0.05).

positive cells was scored as either 0 (0–25%), 1 (26–50%), 2 (51–75%), or 3 (76–100%). The product of these percentage and intensity scores was then used as a final staining score [21], which indicated either 0–3 was considered no or low, 4–9 was considered high expression. The hCG and LHCGR antibody were bought from Abcam Biotechnology (Abcam, Cambridge, UK). The secondary antibody was bought from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.3. qRT-PCR analysis of ovarian tumors

The total RNA was extracted from sample tissues using a Trizol reagent (Invitrogen, Thermo Fisher, USA) according to the manufacturer's instructions, before 1 μg of the total RNA was reverse transcribed using a Reverse Transcription Kit (K1622,Thermo Fisher Scientific, Waltham, MA, USA). hCG, LHCGR, and 18S (endogenous control) mRNA levels were then measured by qRT-PCR, using a real-time PCR system (Roche Cobaz 480, Basel, Switzerland), SYBR Green I Master Mix (Roche, 4887352001, Switzerland), and a LightCycle 480 instrument (Roche Cobaz 480, Switzerland). The PCR cycling program consisted of denaturation at 95 ℃ for 15 s, followed by annealing at 60 for 30 s, and extension of the primer at 72 ° ℃for 30 s, for a total of 45 cyclies. The primers used to amplify each gene comprised an hCG forward (5′-CTA CTG CCC CAC CAT GAC C-3′) and reverse primer (5′-ATG GAC TCG AAG CGC ACA TC-3′), an LHCGR forward (5′-GAA ATG GAT TTG AAG AAG TAC AAA G-3′) and reverse primer (5′−CCA TTG TGC ATC TTC TCC AG-3′), and an 18S rRNA gene forward (5′-GTA ACC CGT TGA ACC CCA TT-3′) and reverse primer (5′−CCA TCC AAT CGG TAG TAG CG-3′). Melting curves were used to evaluate the rate of non-specific amplification, and relative expression levels were calculated using the 2™ΔΔCt method.

2.4. Western blot analyses

Frozen ovarian cancer tissues were rapidly lysed (on ice) using a homogenization buffer (1% NP-40, 50 mmol/l Tris, pH 7.5, 5 mmol/l EDTA, 1% sodium dodecyl sulphate (SDS), 1% sodium deoxycholate, 1% Triton X-100, 1 mmol/l PMSF, 10 mg/ml aprotinin, and 1 mg/ml leupeptin). Lysates were resolved via SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto a PVDF membrane. The membranes were blocked (2 h) in 5% milk (in ddH_2O) and immunoblotted (overnight, 4 ˚C) with hCG (1:500 dilution) and LHCGR (1:500 dilution) antibodies. They were then washed (5 min) in TBST (20 mM Tris, 150 mM NaCl, 0.05% Tween-20) three times and incubated (2 h, room temperature) with horseradish peroxidase-conjugated secondary antibodies (1:5000 dilution). Protein signals were finally visualized using the enhanced chemiluminescence western blotting system (Pierce Company, Woburn, MA, USA). The gray value of the protein is measured by Image J software (NIH, USA).

2.5. Statistical analyses

Chi-square tests were performed to evaluate whether the target protein and mRNA expression levels were correlated with patient clinical or pathological parameters, and/or patient prognoses. The Kaplan–Meier method was used to calculate patient survival curves. The Cox hazard regression method was used for univariate and multivariate analyses. For all analyses, a P-value < 0.05 was considered to indicate statistical significance. All data were analyzed using SPSS17.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. hCG and LHCGR expression levels in fresh tissue samples collected from patients with EOC

hCG and LHCGR mRNA and protein expression levels in EOC were evaluated by analyzing 18 fresh EOC, and five normal (noncancerous) ovarian tissue samples. The demographic characteristic of patients was exhibited in supplemental S1 and S2. The generated data revealed an almost 2-fold upregulation of both hCG mRNA and protein expression in the cancerous ovarian tissue samples ($P < 0.05$) (Fig. 1A, B, C), and conversely, an almost 2-fold decrease in LHCGR mRNA and protein expression in cancerous compared to that in normal ovarian tissue samples ($P < 0.05$) (Fig. 1A, C, E). The IHC staining of hCG and LHCGR in cancer and noncancerous were significantly different (Fig. 1F).

3.2. hCG and LHCGR expression patterns in EOC TMAs

The results of the conducted IHC analyses indicated that hCG protein expression was significantly enhanced in EOC, compared to that in both normal ovarian, or benign ovarian tumor TMAs (Fig. 2). Specifically, high hCG protein expression was observed in 57.55% (80/139) of EOC samples, but in only 0% (0/48), 16.67% (2/12), and 4.65% (2/43) of normal ovarian, benign ovarian tumor, and borderline ovarian cancer samples, respectively ($P < 0.001$) (Table 1). Conversely, LHCGR expression was significantly decreased in EOC compared to that in normal ovarian, and benign ovarian tumor TMAs (Fig. 2), such that low LHCGR expression was observed in 74.82% (104/139) of EOC samples, but in only 22.92% (11/48), 16.67% (2/12), and 30.23% (13/42) of normal ovarian, benign ovarian tumor, and borderline ovarian cancer tissue samples ($P < 0.001$) (Table 2). hCG and LHCGR expression levels were also found to vary between tissue samples taken from ovarian cancers with different histological classifications (Fig. 3).

3.3. Association of hCG and LHCGR expression patterns with patient clinicopathological parameters

Statistical analyses revealed that hCG expression was significantly higher in advanced (III/IV) than in early (I/II) stage EOC, and high hCG expression levels were significantly associated with exhibited patient FIGO tumor stages ($P < 0.001$), and rates of metastasis ($P < 0.001$). In contrast, hCG expression levels did not vary significantly with patient age at diagnosis ($P = 0.259$), tumor grade ($P = 2.921$), tumor histological classification ($P = 0.529$), ascites accumulation ($P = 0.479$), nor serum CA-125 levels ($P = 0.765$) (Table 3). Conversely, LHCGR expression was significantly decreased in advanced (III/IV) compared with early (I/II) stage EOC, and thus, low LHCGR expression levels were significantly associated with more advanced patient FIGO stages ($P < 0.001$), and higher rates of metastasis ($P < 0.001$). LHCGR expression levels did not vary significantly with patient age ($P > 0.999$), tumor grade ($P = 0.137$), histological classification ($P = 0.710$), ascites accumulation ($P = 0.843$), nor serum CA-125 levels ($P = 0.493$) (Table 4). Thus, these results indicate that high hCG and low LHCGR expression levels are both associated with EOC progression.

Generated expression data were also analyzed to evaluate a potential correlation between hCG and LHCGR expression levels in ovarian cancer tissues; the results showed an obvious negative correlation between the hCG and LHCGR ($R = -0.249$, $P = 0.003$) (Fig. 4).

3.4. High hCG and low LHCGR expression levels show poor EOC prognosis

According to the conducted univariate survival analyses, a high level of hCG expression (OR 4.099; $P < 0.001$), more advanced FIGO stages (OR 2.913; $P < 0.001$), and rates of metastasis (OR 3.786; $P < 0.001$) were all factors associated with a reduced overall survival (OS) rate among patients with EOC (Table 5), as were a reduced level of LHCGR expression (OR 0.320; $P = 0.001$), advanced FIGO stages (OR 2.913; $P < 0.001$) and rates of metastasis (OR 3.786; $P < 0.001$) (Table 6). Further examination of these findings (via conducted multivariate analyses) showed that increased hCG expression (HR 3.785; $P < 0.001$) and rates of tumor metastasis (HR 2.619; $P = 0.019$) were

unfavorable prognostic factors for patient OS, independent of other clinicopathological factors (Table 5). Similarly, the multivariate COX regression model was used to demonstrate that LHCGR expression (HR 0.320; P = 0.001) was a favorable prognostic factor for patient OS, independent of other clinicopathological factors (Table 6). Finally, patients who exhibited tumors with high or low levels of hCG and LHCGR expression, respectively, experienced a worse OS rate than those who exhibited tumors with low hCG, or high LHCGR expression levels (Fig. 5).

4. Discussion

Over the last three decades, the 5-year OS rate for ovarian cancer has remained at 38–46% [22]. The majority of patients with ovarian cancer are still diagnosed at advanced stages of the disease, due to its often occult onset; thus, it is essential to develop novel indicators to facilitate its early detection and diagnosis, and effective prognosis.

In recent studies, β-hCG was found to be associated with a poor prognosis, and increased rate of metastasis in various malignant tumors, such as bladder, osteoblastoma, endometrial, colon, lung, and testicular tumors, and furthermore, has been suggested to regulate ovarian cancer metastasis by controlling EMT and apoptosis [9,10,23,24]. Its receptor, LHCGR, is well established to critically regulate ovulation, and to be essential for follicular maturation, ovulation, and corpus luteum function [25]. The LHCGR has also been shown to be expressed in a range of normal nongonadal tissues (including bovine, porcine, rat, mouse, rabbit, and human uterine, adrenal, bone, fallopian tube, brain, retinal, skin, placental, seminal vesicle, prostate, female breast, and thyroid tissues), and its expression has been found to be upregulated in granulosa cells (GC) in women with polycystic ovary syndrome (PCOS) [16,26–28]. Furthermore, the LHCGR is also expressed by several tumor types, including endometrial and breast tumors [8,12,13]. Some previous reports suggested that the interaction between hCG and LHCGR in endometrial cancer tissues may stimulate cell growth and promote neovascularization [29]; however, others have proposed that the effect of hCG on ovarian cancer cells is likely LHCGR-independent [10].

In fact, the effect of LHCGR in tumors, to date, remains controversial. For example, it was previously demonstrated that human endometrial cancers exhibit increased LHCGR expression compared to normal tissues [12], and LH-mediated LHCGR activation has been suggested to contribute to the etiology and/or progression of ovarian cancer [16,17,30]. In addition, LHCGR mRNA expression levels have been shown to be both significantly enhanced, and negatively correlated with LH serum levels in patients with ovarian cancer, (although the latter finding was determined via a study that analyzed a small cohort of patients over several decades) [31]. In contrast, various other studies have suggested that LHCGR is likely associated with anti-oncogenic activities; for example, its expression has been found to be higher in normal breast than in breast-cancer tissues [32].

To date, few studies have investigated whether LHCGR and hCG expression levels correlate in ovarian cancer tissues. Szczerba et al. [10] did previously demonstrate an inverse association between increased hCG levels and lowered LHCGR protein expression levels in an OVCAR3 ovarian-cancer cell model; however, this was not statistically significant. Nevertheless, these findings were consistent with previously generated low-density-array data, which supports the hypothesis that LH-mediated LHCGR activation decreases the invasiveness and migration of ovarian cancer cells [33].

Based on these collected findings, the present study investigated whether hCG and LHCGR expression levels were correlated in tissue samples from 139 patients with EOC, and/or whether they were associated with patient clinicopathological characteristics. The results of the conducted RT-PCR analyses revealed significantly increased and decreased hCG and LHCGR mRNA levels, respectively, in cancerous compared to those in normal ovarian tissues, and these findings were supported by the results of the conducted western blot analyses. Furthermore, high hCG expression was shown to be associated with EOC progression and a worsened patient prognosis, as indicated by more advanced FIGO tumor stages, and increased rates of tumor metastasis. Similarly, low LHCGR expression level was found to correlate with advanced FIGO tumor stages, and increased rates of tumor metastasis. However, numerous studies have shown that the different histological types of ovarian carcinoma are profoundly different diseases, our studies found that the expression of hCG or LHCGR was not significantly associated with the histological types of EOC. Moreover, the OS rate experienced by patients that exhibited tumors with high hCG, or low LHCGR expression levels was lower than that experienced by patients that exhibited tumors with low hCG or high LHCGR expression. Thus, hCG and LHCGR expression levels may be promising potential prognostic markers for patients with EOC, given that they correlate consistently with patient disease stages, rates of metastasis, and OS.

hCG may affect the expression of LHCGR in luteal cells, with a decrease in receptors reducing the binding activity of hCG [34]. However, the present study found a significant negative relationship between hCG and LHCGR expression levels in the analyzed tissue samples, suggesting that mechanisms other than LHCGR may regulate hCG activity in this context.

In conclusion, increased hCG expression and low LHCGR expression levels in ovarian cancer tissues are positively associated with ovarian cancer progression and metastasis, and thereby, with a worsened patient OS rate. Thus, both hCG and LHCGR should be investigated as potential novel markers or therapeutic targets for ovarian cancer. For example, LHCGR-targeted therapeutic agents may be a promising approach to reduce mortality and morbidity in ovarian cancer, while incurring less toxic effects in patients. Further research is needed to both elucidate the mechanisms by which hCG and LHCGR modulate ovarian cancer progression and evaluate their potential clinical value as therapeutic targets.

Conflict of interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

[1] J. Li, K. Hu, G. Gong, D. Zhu, Y. Wang, H. Liu, X. Wu, Upregulation of MiR-205 transcriptionally suppresses SMAD4 and PTEN and contributes to human ovarian cancer progression, Sci. Rep. 7 (2017) 41330, https://doi.org/10.1038/srep41330.
[2] B. Mateescu, L. Batista, M. Cardon, T. Gruosso, Y. de Feraudy, O. Mariani, A. Nicolas, J.P. Meyniel, P. Cottu, X. Sastre-Garau, F. Mechta-Grigoriou, miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response, Nat. Med. 17 (2011) 1627–1635, https://doi.org/10.1038/nm.2512.
[3] Y. Sun, L. Hu, H. Zheng, M. Bagnoli, Y. Guo, R. Rupaimoole, C. Rodriguez-Aguayo, G. Lopez-Berestein, P. Ji, K. Chen, A.K. Sood, D. Mezzanzanica, J. Liu, B. Sun, W. Zhang, MiR-506 inhibits multiple targets in the epithelial-to-mesenchymal transition network and is associated with good prognosis in epithelial ovarian cancer, J. Pathol. 235 (2015) 25–36, https://doi.org/10.1002/path.4443.
[4] J. Li, M. Yin, W. Song, F. Cui, W. Wang, S. Wang, H. Zhu, B subunit of human chorionic gonadotropin promotes tumor invasion and predicts poor prognosis of early-stage colorectal cancer, Cell. Physiol. Biochem. 45 (2018) 237–249, https://doi.org/10.1159/000486770.
[5] S. Schüler-Toprak, O. Treeck, O. Ortmann, Human chorionic gonadotropin and breast cancer, Int. J. Mol. Sci. 18 (2017) 1587, https://doi.org/10.3390/ijms18071587.
[6] R. Nishimura, T. Koizumi, K. Morisue, N. Yamanaka, R. Laiwani, M. Yoshimura,

T. Nakagawa, K. Shil, K. Hasegawa, S. Baba, Expression and secretion of the beta subunit of human chorionic gonadotropin by bladdercarcinoma in vivo and in vitro, Cancer Res. 55 (1995) 1479–1484.
[7] Y. Konishi, F. Kawamata, H. Nishihara, S. Homma, Y. Kato, M. Tsuda, S. Kohsaka, T. Einama, C. Liu, T. Yoshida, A. Nagatsu, M. Tanino, S. Tanaka, H. Kawamura, T. Kamiyama, A. Taketomi, Tumor budding and human chorionic gonadotropin-beta expression correlate with unfavorable patient outcome in colorectal carcinoma, Med. Oncol. 35 (104) (2018), https://doi.org/10.1007/s12032-018-1164-x.
[8] A. Jankowska, M. Andrusiewicz, J. Grabowski, E. Nowak-Markwitz, J.B. Warchol, Coexpression of human chorionic gonadotropin beta subunit and its receptor in nontrophoblastic gynecological cancer, Int. J. Gynecol. Cancer 18 (2008) 1102–1107, https://doi.org/10.1111/j.1525-1438.2007.01151.x.
[9] N. Liu, S.M. Peng, G.X. Zhan, J. Yu, W.M. Wu, H. Gao, X.F. Li, X.Q. Guo, Human chorionic gonadotropin beta regulates epithelial-mesenchymal transition and metastasis in human ovarian cancer, Oncol. Rep. 38 (2017) 1464–1472, https://doi.org/10.3892/or.2017.5818.
[10] A. Szczerba, A. ŚLiwa, M. Kubiczak, E.W.A. Nowak-Markwitz, A. Jankowska, Human chorionic gonadotropin β subunit affects the expression of apoptosis-regulating factors in ovarian cancer, Oncol. Rep. 35 (2016) 538–545, https://doi.org/10.3892/or.2015.4386.
[11] M.A. Nieto, The ins and outs of the epithelial to mesenchymal transition in health and disease, Annu. Rev. Cell Dev. Biol. 27 (2011) 347–376.
[12] J. Lin, Z.M. Lei, S. Lojun, C.V. Rao, P.G. Satyaswaroop, T.G. Day, Increased expression of luteinizing hormone/human chorionic gonadotropin receptor gene in human endometrial carcinomas, J. Clin. Endocrinol. Metab. 79 (1994) 1483–1491.
[13] F. Sorbi, E. Projetto, I. Turrini, G. Baroni, S. Pillozzi, V. Ghizzoni, F. Vergoni, F. Castiglione, F. Malentacchi, M. Fambrini, I. Noci, Luteinizing Hormone/Human chorionic gonadotropin receptor immunohistochemical score associated with poor prognosis in endometrial cancer patients, Biomed Res. Int. 2018 (2018) 1618056, , https://doi.org/10.1155/2018/1618056.
[14] I. Noci, S. Pillozzi, E. Lastraioli, S. Dabizzi, M. Giachi, E. Borrani, J. Wimalasena, G.L. Taddei, G. Scarselli, A. Arcangeli, hLH/hCG-receptor expression correlates with in vitro invasiveness in human primary endometrial cancer, Gynecol. Oncol. 111 (2008) 496–501, https://doi.org/10.1016/j.ygyno.2008.08.018.
[15] M. Ascoli, F. Fanelli, D.L. Segaloff, The lutropin/choriogonadotropin receptor, a 2002 perspective, Endocr. Rev. 23 (2002) 141–174.
[16] J.H. Choi, A.S. Wong, H.F. Huang, P.C. Leung, Gonadotropins and ovarian cancer, Endocr. Rev. 28 (2007) 440–461.
[17] P.C. Leung, J.H. Choi, Endocrine signaling in ovarian surface epithelium and cancer, Hum. Reprod. Update 13 (2007) 143–162.
[18] I. Huhtaniemi, Are gonadotrophins tumorigenic–a critical review of clinical and experimental data, Mol. Cell. Endocrinol. 329 (2010) 56–61, https://doi.org/10.1016/j.mce.2010.04.028.
[19] M. Su, W. Wei, X. Xu, W. Xiaoying, C. Caoyi, S. Li, Z. Yuquan, Role of hCG in vasculogenic mimicry in OVCAR-3 ovarian cancer cell line, Int. J. Gynecol. Cancer 21 (2011) 1366–1374, https://doi.org/10.1097/IGC.0b013e31822c7529.
[20] W.L. Yang, Z. Lu, R.C. Bast Jr, The role of biomarkers in the management of epithelial ovarian cancer, Expert Rev. Mol. Diagn. 17 (2017) 577–591.
[21] Y. Xu, C. Wang, Y. Zhang, L. Jia, J. Huang, Overexpression of MAGE-A9 is predictive of poor prognosis in epithelial ovarian cancer, Sci. Rep. 5 (2015) 12104, https://doi.org/10.1038/srep12104.
[22] S. Capriglione, D. Luvero, F. Plotti, C. Terranova, R. Montera, G. Scaletta, T. Schiro, G. Rossini, P. Benedetti Panici, R. Angioli, Ovarian cancer recurrence and early detection: may HE4 play a key role in this open challenge? A systematic review of literature, Med. Oncol. 34 (2017) 164, https://doi.org/10.1007/s12032-017-1026-y.
[23] C.D. Morris, M.R. Hameed, N.P. Agaram, S. Hwang, Elevated beta-hCG associated with aggressive Osteoblastoma, Skeletal Radiol. 46 (2017) 1187–1192, https://doi.org/10.1007/s00256-017-2647-0.
[24] Q. Ji, P. Chen, C. Aoyoma, P. Liu, Increased expression of human luteinizing hormone/human chorionic gonadotropin receptor mRNA in human endometrial cancer, Mol. Cell. Probes 16 (2002) 269–275.
[25] J.S. Richards, M. Ascoli, Endocrine, paracrine, and autocrine signaling pathways that regulate ovulation, Trends Endocrinol. Metab. 29 (2018) 313–325, https://doi.org/10.1016/j.tem.2018.02.012.
[26] V. Kanamarlapudi, U.D. Gordon, A. Lopez Bernal, Luteinizing hormone/chorionic gonadotrophin receptor overexpressed in granulosa cells from polycystic ovary syndrome ovaries is functionally active, Reprod. Biomed. Online 32 (2016) 635–641, https://doi.org/10.1016/j.rbmo.2016.03.003.
[27] A.L. Frazier, L.S. Robbins, P.J. Stork, R. Sprengel, D.L. Segaloff, R.D. Cone, Isolation of TSH and LH/CG receptor cDNAs from human thyroid: regulation by tissue specific splicing, MolecularEndocrinology 4 (1990) 1264–1276.
[28] H.E. Carlson, P. Kane, Z.M. Lei, X. Li, C.V. Rao, Presence of luteinizing hormone/human chorionic gonadotropin receptors in male breast tissues, J. Clin. Endocrinol. Metab. 89 (2004) 4119–4123.
[29] A.G. Jankowska, M. Andrusiewicz, N. Fischer, P.J. Warchol, Expression of hCG and GnRHs and their receptors in endometrial carcinoma and hyperplasia, Int. J. Gynecol. Cancer 20 (2010) 92–101, https://doi.org/10.1111/IGC.0b013e3181bbe933.
[30] M. Mandai, I. Konishi, H. Kuroda, S. Fujii, LH/hCG action and development of ovarian cancer–a short review on biological and clinical/epidemiological aspects, Mol. Cell. Endocrinol. 269 (2007) 61–64.
[31] N.M. El-Etreby, A.A. Ghazy, R. Rashad, Prohibitin: targeting peptide coupled to ovarian cancer, luteinization and TGF-beta pathways, J. Ovarian Res. 10 (2017) 28, https://doi.org/10.1186/s13048-017-0325-4.
[32] T.M. Kuijper, K. Ruigrok-Ritstier, M. Verhoef-Post, D. Piersma, M.W. Bruysters, E.M. Berns, A.P. Themmen, LH receptor gene expression is essentially absent in breast tumor tissue: implications for treatment, Mol. Cell. Endocrinol. 302 (2009) 58–64, https://doi.org/10.1016/j.mce.2008.12.016.
[33] J. Cui, B.M. Miner, J.B. Eldredge, S.W. Warrenfeltz, P. Dam, Y. Xu, D. Puett, Regulation of gene expression in ovarian cancer cells by luteinizing hormone receptor expression and activation, BMC Cancer 11 (2011) 280, https://doi.org/10.1186/1471-2407-11-280.
[34] Y.M. Hoffman, H. Peegel, M.J. Sprock, Z. Qingyu, K.M. Menon, Evidence that human chorionic gonadotropin/luteinizing hormone receptor down-regulation involves decreased levels of receptor messenger ribonucleic acid, Endocrinology 128 (1991) 388–393.