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Differential expression of estrogen receptor subtypes in ovarian high-grade serous carcinoma and clear cell carcinoma

Daiken Osaku | Tetsuro Oishi | Naoshi Kawamura | Yuki Iida | Hiroaki Komatsu | Akiko Kudoh | Jun Chikumi | Shinya Sato | Tasuku Harada

Department of Obstetrics and Gynecology, Tottori University School of Medicine, Yonago, Japan

Correspondence
Tetsuro Oishi, Department of Obstetrics and Gynecology, Tottori University School of Medicine 36-1 Nishicho, Yonago, Tottori prefecture 683-8504, Japan.
Email: tetsuro@tottori-u.ac.jp

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Abstract

Purpose: To investigate the role of estrogen receptors (ERs) in high-grade serous carcinoma (HGSC) and clear cell carcinoma (CCC) of the ovary and evaluate ERs as prognostic biomarkers for ovarian cancer.

Methods: This study included 79 patients with HGSC (n = 38) or CCC (n = 41) treated at our institution between 2005 and 2014. Immunohistochemistry examined protein expression of ERα, ERβ, and G protein-coupled estrogen receptor-1 (GPER-1); relationships between ERα, ERβ, and GPER-1 with patient survival were evaluated. Additionally, cell proliferation assay and phosphokinase proteome profiling were performed.

Results: In HGSC patients, expression of ERα, cytoplasmic GPER-1, or nuclear GPER-1 was associated with poor progression-free survival (PFS) ($P = .041$, $P = .010$, or $P = .013$, respectively). Cytoplasmic GPER-1 was an independent prognostic factor for PFS in HGSC patients (HR = 2.83, 95% CI = 1.03-9.16, $P = .007$). ER expressions were not associated with prognosis in CCC patients. GPER-1 knockdown by siRNA reduced the cells number to 60% of siRNA-control-treated cells ($P < .05$), and GPER-1 antagonist, G-15 inhibited two HGSC cell lines proliferation (KF and UWB1.289) in a dose-dependent manner. Phosphoprotein array revealed that GPER-1 silencing decreased relative phosphorylation of glycogen synthase kinase-3.

Conclusions: High GPER-1 expression is an independent prognostic factor for PFS in HGSC patients, and GPER-1 may play a role in HGSC cell proliferation.

KEYWORDS

G protein-coupled estrogen receptor-1, high-grade serous carcinoma of the ovary, ovarian clear cell carcinoma, progression-free survival, proliferation

1 | INTRODUCTION

Globally, more than 240 000 women are newly diagnosed with ovarian cancer every year. The 5-year survival rate in all stages of the disease is approximately 45%.[1] In Japan, 13 345 women were newly diagnosed with ovarian cancer, and 4733 people died of ovarian cancer in 2019.[2] High-grade serous carcinoma of the ovary (HGSC) is the most common histologic subtype of epithelial ovarian cancer (EOC) and is

often found at an advanced stage, thus leading to a worse prognosis. In addition, clear cell carcinoma of the ovary (CCC) is endometriosis-associated ovarian cancer and is the second most common histologic subtype of EOC,[2] and its clinical features are distinctly different from those of HGSC. The poor prognosis of patients with advanced CCC may be caused by resistance to conventional chemotherapy.[3,4]

Estrogens play an essential role in ovarian carcinogenesis,[5] and its biological effects are mediated by estrogen receptors (ERs), such as ERα, ERβ, and G protein-coupled estrogen receptor-1 (GPER-1)/ G protein-coupled receptor 30 (GPR30). ERα and ERβ function as transcription factors.[6,7] A previous study showed that ERα and progesterone receptors were prognostic factors in patients with HGSC.[8] In contrast, CCC is known to have negative ERα expression.[8,9] We previously demonstrated that mRNA expression of ERα in endometriotic cells is lower than that in endometrial cells, whereas mRNA expression of ERβ is detected in both cells.[10] Therefore, ERβ may play a role in the development of CCC, one histological subtype of endometriosis-associated ovarian cancers. However, few studies have investigated the relationship between ERβ and CCC. GPER-1, which was first identified in 1996, is a seven-transmembrane domain receptor localized on the cell surface.[11] GPER-1, originally named GPR30, has a high affinity for estrogen.[12] The prognostic value of GPER-1 in EOC remains controversial. A variety of studies have reported the oncogenic features of the GPER-1 gene and protein. For example, Smith et al[13] showed an association of high GPER-1 expression with a lower survival rate in patients with ovarian cancer, and Fujiwara et al found an association of both GPER-1 expression and EGFR expression with poor outcomes in ovarian cancer.[14] In contrast, Ignatov et al[15] demonstrated a relation between low GPER-1 expression and favorable outcomes in EOC, and Schuler-Toprak et al[16] observed longer overall survival (OS) and progression-free survival (PFS) in ovarian cancer patients with high mRNA expression of GPER-1 using open-access data. However, the association between GPER-1 and patient survival in CCC has not been proved, probably because an extremely low number of patients with this histologic subtype were included in those previous studies.[13,14] Therefore, the relationship between GPER-1 expression and prognosis is still unclear in patients with CCC.

Accordingly, we investigated the function of ERs in HGSC and CCC and assessed their utility as prognostic biomarkers for ovarian cancer. Our findings suggest that high GPER-1 expression is an independent prognostic factor for PFS in patients with HGSC.

2 | MATERIALS AND METHODS

2.1 | Patients

This retrospective study recruited a total of 79 patients who had one of the two histologic subtypes of primary EOC (HGSC [n = 38] or CCC [n = 41]) and were treated at the Tottori University Hospital between 2005 and 2014. These patients underwent surgical staging and cytoreduction, followed by chemotherapy. Written informed consent was obtained from all patients according to the institutional guidelines. We collected tumor samples from cancer tissues during surgery and stored them as formalin-fixed paraffin-embedded tissues. We reviewed archived medical records to obtain data on patient demographics, tumor characteristics, treatment types, and survival. This study was approved by the Institutional Review Board of Tottori University Hospital (IRB number 19-A198).

2.2 | Immunohistochemistry

Immunohistostaining was performed as previously described.[17] We used rabbit polyclonal antibodies against ERα (clone SP1; dilution: 1:150; Abcam), ERβ (clone 14C8; dilution: 1:150; Abcam), or GPER-1 (clone A-20; dilution: 1:150; Abcam). For the negative control, phosphate-buffered saline was used instead of the primary antibodies. We used the MCF7 cell line as the positive control for ERα, ERβ, and GPER-1. The immunoreactive score (IRS) was used to evaluate staining of cancer cells, as described by Remmele and Stegner.[18]

For GPER-1, we evaluated cytoplasmic and nuclear staining of tumor tissues using IRS based on a previous study.[19] We used the median IRS score as the cutoff to determine high expression (IRS ≥6) versus low expression (IRS <6).

2.3 | Cell lines

Two human HGSC cell lines, KF, and UWB1.289 were used in this study. KF was obtained from Dr Yoshihiro Kikuchi (National Defense Medical College) and was maintained in phenol red-free Dulbecco's modified Eagle's medium/Ham's F12 (Nacalai Tesque) with charcoal-treated fetal calf serum (FCS), 100 units/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere containing 5% CO_2 at 37°C. UWB1.289 was obtained from the American Type Culture Collection (ATCC; Rockville) and was maintained in 50% ATCC-formulated RPMI-1640 medium and 50% mammary epithelial growth medium (Clonetics/Lonza, Walkersville) with the same medium supplements under the same condition as KF cells.

2.4 | Short interfering RNA (siRNA) transfection

Cells were seeded in 6-well culture plates at 8.0×10^4 cells/well (30%-50% confluence). The next day, cells were transfected with Stealth siRNAs against GPER-1 or a negative control hi GC (Thermo Fisher Scientific) at a final siRNA concentration of 50 pmol/L using Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific) for 24 hours.

2.5 | Evaluation of gene expression of ERα, Erβ, and GPER-1

Messenger RNA (mRNA) expression of ERα, ERβ, and GPER-1 was determined by real-time reverse transcription-polymerase chain

TABLE 1 Patient characteristics

		HGSC (n = 38)	CCC (n = 41)	*P*-values
Age in years, median (range)		62 (40-77)	58 (35-85)	.117
FIGO stage	I	3	19	<.0001
	II	2	7	
	III	24	12	
	IV	9	3	
Residual disease	<1.0 cm	32	36	.753
	≥1.0 cm	6	5	
Duration of follow-up days, median (range)		1311 (287-2850)	1587 (110-4481)	.010

Abbreviations: CCC, clear cell carcinoma of the ovary; FIGO, International Federation of Gynecology and Obstetrics; HGSC, high-grade serous carcinoma of the ovary.

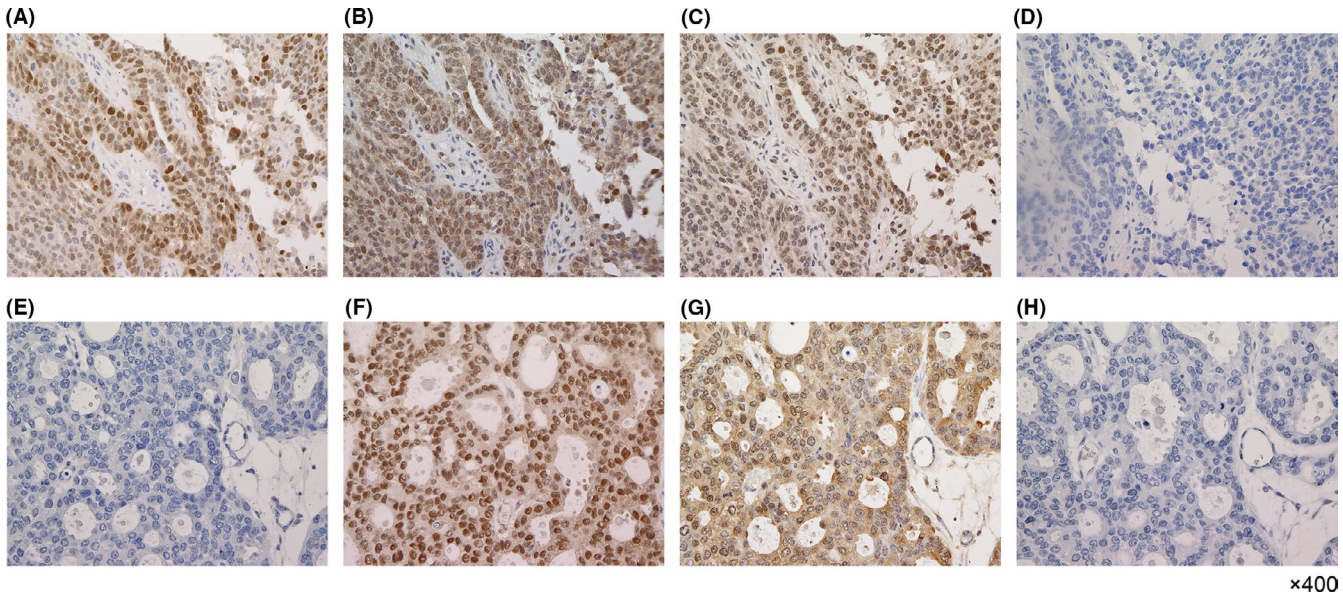

FIGURE 1 Representative images of immunohistochemical staining (Original magnification: 400×). High-grade serous carcinoma of the ovary: A, estrogen receptor α; B, estrogen receptor β; C, G protein-coupled estrogen receptor-1; and D, negative control. Ovarian clear cell carcinoma: E, estrogen receptor α (IRS score: 0); F, estrogen receptor β; G, G protein-coupled estrogen receptor-1; and H, negative control. IRS score for all images except for panel "e" were 12 [Colour figure can be viewed at wileyonlinelibrary.com]

reaction (RT-PCR). Total RNA was isolated from cultured cells using an RNeasy Mini Plus Kit (Qiagen). Complementary DNA was synthesized from the isolated RNA by RT with the High Capacity cDNA Reverse Transcription Kit ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo). Primers for the target genes and protocols have been previously described.[10] In addition, evaluation of GPER-1 gene expression after GPER-1 RNAi was performed using TaqMan PCR assay probes (Applied Biosystems). Glyceraldehyde 3-phosphate dehydrogenase was used as a reference gene.

2.6 | Phosphokinase proteome profiling

After transfection of GPER-1 siRNA, the protein expression profiles were examined using the Phospho-Kinase Array (ARY003B®, R&D Systems Inc.). Phosphokinase signals were detected using X-ray films following exposure to chemiluminescent reagents. The array was visualized using a ChemiDoc Touch MP (Bio-Rad Laboratories Inc.). We quantified protein phosphorylation levels using a pixel density module in ImageJ.

2.7 | Cell proliferation assay

The effect of GPER-1 silencing on cell growth after transfection with GPER-1 siRNA was examined using the WST-1 assay. After 24-h incubation with siRNA, cells were incubated with a fresh medium for 24 hours. The next day, the cells were harvested and seeded at 2×10^4/well in 96-well plates. The proliferative potential of cells was analyzed using a Cell Counting Kit® (Dojindo) after 48-h incubation.

Additionally, GPER-1 agonist G1 and antagonist G15 (Cayman Chemical) were used to examine the effect of GPER-1 on cell

proliferation. The cells were seeded at a density of 1×10^4/well in 96-well plates. The next day, various concentrations of G1 or G15 (0.1, 1, 5, and 10 μmol/L) were added. After 24-hours incubation with these reagents, cell proliferation was examined using the WST-1 assay.

2.8 | Statistical analyses

All statistical analyses were performed using JMP version 11 (SAS Institute Inc.). Data were analyzed using Fisher's exact test or the Mann-Whitney *U* test. We calculated PFS and OS using the Kaplan-Meier method. The log-rank test was used for comparison of survival curves in each group. Multivariate analysis was performed to fit the Cox proportional hazards model. Statistical significance was set at $P < .05$.

3 | RESULTS

A total of 79 patients with International Federation of Gynecology and Obstetrics (FIGO) stages I to IV HGSC or CCC were enrolled in our study. Demographic and clinicopathologic data of these 79 patients with HGSC (n = 38) or CCC (n = 41) are presented in Table 1. Generally, there were more early cases of CCC and more advanced cases of HGSC. Therefore, the follow-up time was slightly longer in HGSC than in CCC. There were no differences in age distribution and residual disease after cytoreductive surgery between the two groups.

High ERα expression was more frequent in HGSC than in CCC (55.2% vs 3.7%, $P < .001$; Figure 1). The frequency of high expression of ERβ, cytoplasmic GPER-1, and nuclear GPER-1 was comparable between HGSC and CCC (ERβ: 52.6% vs 63.4%, $P = .2643$; cytoplasmic GPER-1:55.2% vs. 53.6%, $P = .62$; nuclear GPER-1:36.1% vs 58.8%, $P = .09$). In patients with HGSC, expression of ERα, cytoplasmic GPER-1, or nuclear GPER-1 was associated with poor PFS (ERα, $P = .041$; cytoplasmic GPER-1, $P = .010$; nuclear GPER-1, $P = .013$), and ERα and nuclear GPER-1 expression could be used to predict OS for HGSC (Erα, $P = .035$; nuclear GPER-1, $P = .035$; Table 2 and Figure 2). In patients with CCC, there was no relationship between ER expression and patient survival (Table 2). Multivariate analysis revealed that cytoplasmic GPER-1 expression was an independent prognostic factor for PFS in HGSC patients (HR = 2.83, 95% CI = 1.03-9.16, $P = .007$; Table 2).

We examined mRNA expression of ERα, ERβ, and GPER-1 in seven HGSC cell lines and found that GPER-1 mRNA was

TABLE 2 Univariate and multivariate analyses (HGSC and CCC)

(A) Progression-free survival (HGSC)						
			Univariate analysis		Multivariate analysis	
Factors		n	Median PFS (days)	*P*-values	HR(95% CI)	*P*-values
Age				.165		
	<60	13	1875			
	≥60	25	988			
FIGO stage				.0205	2.792e+9 (3.57-2.60e+64)	.0007
	I-II	6	2144			
	III-IV	32	611			
Residual disease				.2194		
	<1.0cm	32	1875			
	≥1.0cm	6	802			
ERα				.0413	1.34 (0.49-4.10)	.5756
	Low	17	936			
	High	21	568			
ERβ				.2831		
	Low	18	729			
	High	20	643			
GPER-1 in cytoplasm				.007	4.33 (1.05-19.18)	.042
	Low	17	1263			
	High	21	544			
GPER-1 in nucleus				.0133	1.32 (0.46-4.29)	.609
	Low	24	939			
	High	14	527			

(Continues)

TABLE 2 (Continued)

(B) Overall survival (HGSC)						
			Univariate analysis		Multivariate analysis	
Factors		**n**	**Median OS (days)**	***P*-values**	**HR(95% CI)**	***P*-values**
Age				.4777		
	<60	13	1311			
	≥60	25	1991			
FIGO stage				.0184	1.99e+9 (2.72-4.48)	.0021
	I-II	6	2067			
	III-IV	32	1311			
Residual disease				.2469		
	<1.0cm	32	1824			
	≥1.0cm	6	1311			
ERα				.0353	1.85 (0.67-5.70)	.241
	Low	17	2067			
	High	21	1130			
ERβ				.0884		
	Low	18	2067			
	High	20	1308			
GPER-1 in cytoplasm				.0995		
	Low	17	1830			
	High	21	1130			
GPER-1 in nucleus				.0352	2.07 (0.77-5.77)	0.1497
	Low	24	1824			
	High	14	1048			
(C) Progression-free survival (CCC)						
			Univariate analysis		Multivariate analysis	
Factors		**n**	**Median PFS (days)**	***P*-values**	**HR(95% CI)**	***P*-values**
Age				.09	0.58 (0.12-2.06)	.4205
	<60	18	Undefined			
	≥60	23	Undefined			
FIGO stage				<.0001	25.79 (4.84-477)	<.0001
	I-II	15	Undefined			
	III-IV	26	358			
Residual disease				.7591	9.88 (0.37-263)	.1445
	<1.0cm	37	Undefined			
	≥1.0cm	4	2483			
ERα						
	Low	41				
	High	0				
ERβ				.8222		
	Low	26	Undefined			
	High	15	3075			
GPER-1 in cytoplasm				.7150		
	Low	24	Undefined			
	High	17	Undefined			

(Continues)

TABLE 2 (Continued)

(C) Progression-free survival (CCC)						
			Univariate analysis		Multivariate analysis	
Factors		**n**	**Median PFS (days)**	***P*-values**	**HR(95% CI)**	***P*-values**
GPER-1 in nucleus				.6238		
	Low	25	Undefined			
	High	16	Undefined			

(D) Overall survival (CCC)						
			Univariate analysis		Multivariate analysis	
Factors		**n**	**Median OS (days)**	***P*-values**	**HR(95% CI)**	***P*-values**
Age				.1503	0.87 (0.15-3.42)	.8497
	<60	18	Undefined			
	≥60	23	Undefined			
FIGO stage				<.0001	29.97 (5.31-569)	<.0001
	I-II	15	Undefined			
	III-IV	26	923			
Residual disease				.8315	13.51 (0.50-371)	.1058
	<1.0cm	37	Undefined			
	≥1.0cm	4	Undefined			
ERα						
	Low	41				
	High	0				
ERβ				.7345		
	Low	26	Undefined			
	High	15	Undefined			
GPER-1 in cytoplasm				.7372		
	Low	24	Undefined			
	High	17	Undefined			
GPER-1 in nucleus				.6817		
	Low	25	Undefined			
	High	16	Undefined			

Abbreviations: CCC, clear cell carcinoma of the ovary; CI, confidence interval; ER, estrogen receptor; FIGO, international federation of gynecology and obstetrics; GPER-1, G protein-coupled estrogen receptor-1; HGSC, high-grade serous carcinoma of the ovary; HR, hazard ratio; OS, overall survival. PFS, progression-free survival.

similarly expressed in all seven cell lines. As for ERα and ERβ mRNA, KF, SKOV3, and UWB1.289 had positive ERα expression and negative ERβ expression, whereas SHIN3 and TU-OS3 had negative ERα expression and positive ERβ expression (Figure 3A).

We next investigated whether proliferation of HGSC cells with GPER-1 expression is GPER-1 dependent. Similar to the current study, a study using a larger number of specimens also showed strong expression of ERα protein in more than 60% of cases (8). Therefore, we selected cell lines that showed both ERα expression and GPER-1 expression for subsequent experiments. Transfection of KF and UWB1.289 cells with GPER-1 siRNA reduced GPER-1 mRNA levels to approximately 20% of the mRNA levels in negative control cells (Figure 3B). Notably, knockdown of GPER-1 by siRNA reduced the number of cells to 60% of that of negative control cells ($P < .05$; Figure 4A,B).

To confirm the potential role of GPER-1 in HGSC proliferation, we used GPER-1 agonist G-1 and antagonist G-15. G-15 inhibited the proliferation of KF and UWB1.289 cells in a dose-dependent manner (Figure 4C,D). No significant change in proliferation was observed after treatment with G-1 (data not shown).

Protein kinases play a significant role in cell proliferation. We used a phosphokinase array for parallel determination of the relative level of protein kinase phosphorylation. GPER-1 silencing decreased

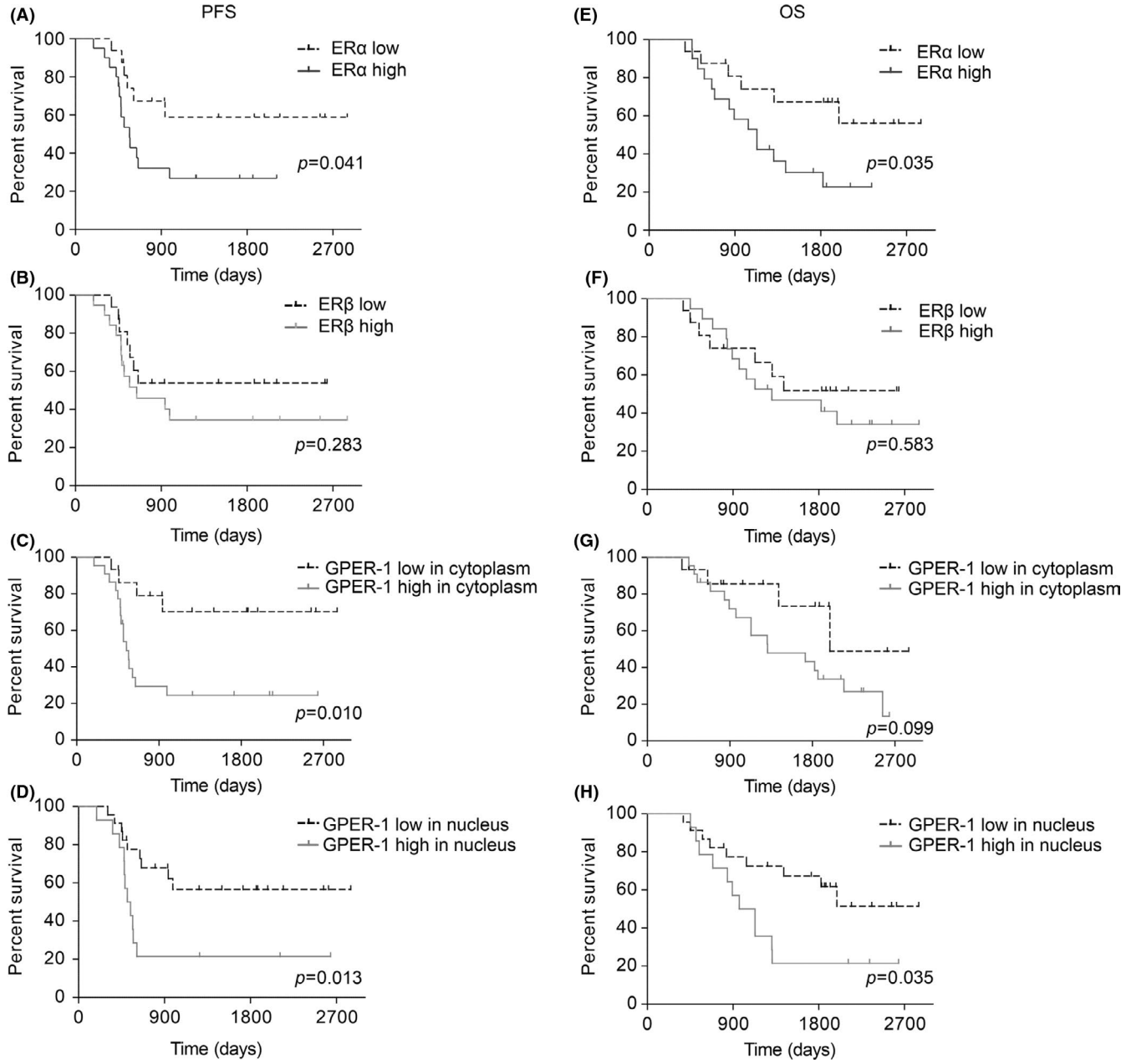

FIGURE 2 Kaplan-Meier curves of progression-free survival (A-D) and overall survival (E-H) in patients with HGSC by estrogen receptor status. Survival by estrogen receptor α (A, E) expression, estrogen receptor β (B, F) expression, cytoplasmic G protein-coupled estrogen receptor-1 (C, G) expression, and nuclear G protein-coupled estrogen receptor-1 (D, H) expression

relative phosphorylation levels of glycogen synthase kinase-3 (GSK--3α/β) and HSP60 in KF cells and those of GSK-3α/β, AKT, ERK1/2, c-Jun, and JNK in UWB 1.289 cells. GSK-3 levels were commonly reduced in the two HGSC cell lines (Figure 4E-G).

4 | DISCUSSION

The present study investigated the expression of estrogen receptors (ERα, ERβ, and GPER-1) in HGSC and CCC tissues. We found an association of high cytoplasmic GPER-1 expression with significantly poor PFS in patients with HGSC, and cytoplasmic GPER-1 expression and FIGO stage were independent prognostic factors for PFS. We also demonstrated the growth inhibitory effect of both GPER-1 knockdown and antagonist on HGSC cells.

A previous study showed that high expression of GPER-1 was observed more frequently in EOC than in borderline tumors (48.3% vs 20%, $P = .002$) and was associated with lower 5-year survival rates.[13] Another study found associations of both GPER-1 expression and EGFR expression with poor PFS in ovarian cancer.[14] These findings are consistent with our observations. However, Ignatov et al and Schüler-Toprak et al demonstrated an association of

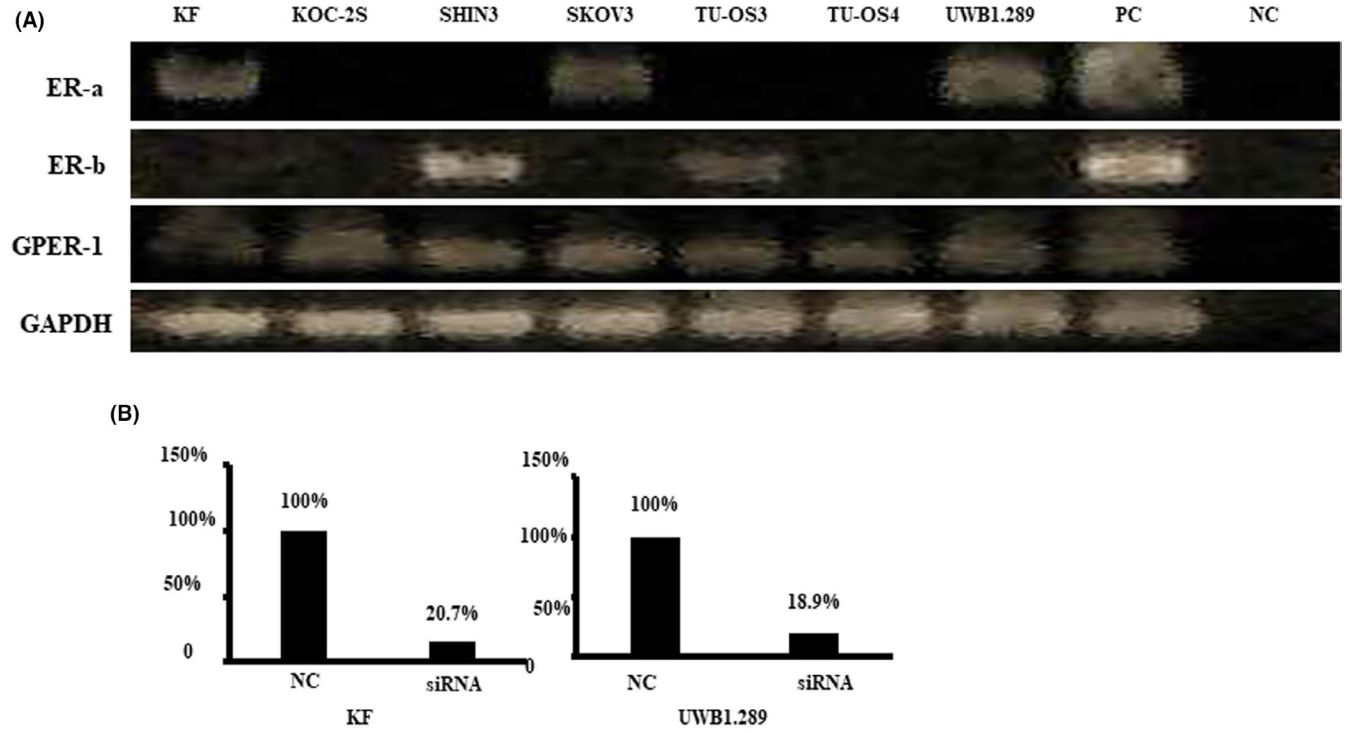

FIGURE 3 mRNA expression of estrogen receptor α, estrogen receptor β, and G protein-coupled estrogen receptor-1 in 7 HGSC cell lines (A). Transfection of KF and UWB1.289 cells with siRNA specific for G protein-coupled estrogen receptor-1 reduced G protein-coupled estrogen receptor-1 mRNA levels to approximately 20% of the levels in siRNA-control-treated cells (B) [Colour figure can be viewed at wileyonlinelibrary.com]

GPER-1 expression with favorable clinical outcomes and suppression of cell proliferation by G-1, a selective GPER-1 agonist[15,16]. The conflicting results in these studies mentioned above may arise from the application of different cell lines and different concentrations of the agonist.

Two previous studies, which included a small number of CCC cases, showed that the relationship between GPER-1 expression and prognosis in CCC was unclear.[13,14] Notably, there were a larger number of CCC cases in this study than in previous studies, and we found that GPER-1 was not associated with prognosis in CCC. Moreover, Akahane et al showed lower ERα expression in atypical endometriosis adjacent CCC than in endometriosis adjacent CCC, and they concluded that loss of hormone dependence might be linked to malignant transformation to CCC.[20] Although the function of GPER-1 without ERα expression is unclear, CCC may become hormone-independent during the process of carcinogenesis, and hormone receptor expression may no longer have an impact on prognosis.

Chan et al[21] found that high nuclear expression of ERβ5 was an independent prognostic factor for OS in EOC. In addition, Ciucci et al showed that ovarian cancer patients with cytoplasmic ERβ2 expression had significantly worse outcomes than those without cytoplasmic ERβ2 expression because of chemoresistance.[22] The present study demonstrated no significant correlation between ERβ expression and patient survival in either HGSC or CCC. Further studies are warranted to elucidate the function of ERβ isoforms in ovarian cancers, especially in CCC.

In the current study, downregulation of GSK-3 was induced by GPER-1 knockdown in ovarian cancer cell lines. Bang et al found that GSK-3α/β promoted proliferation and survival of pancreatic cancer cells.[23] In addition, Cao et al reported cell cycle progression and accelerated cell proliferation induced by overexpression of the constitutively active form of GSK-3β in ovarian cancer cells; however, GSK-3 inhibition prevented tumorigenicity in nude mice.[24] Consistently, Sun et al[25] showed that LiCl, a GSK inhibitor, significantly inhibited cell proliferation, as indicated by reduced DNA replication and cell cycle arrest in prostate cancer cells. The aforementioned findings indicate a possible association between GPER-1, GSK-3, and cell proliferation.

This study has some limitations. First, our study was a retrospective study in a single institution, and the number of patients was small. Further studies with a prospective design and larger sample size are needed to provide more conclusive evidence. Second, we did not investigate the prognostic impact of the interaction between ERα, ERβ isoforms, and GPER-1. The ERs-related signaling pathways are complicated, and crosstalk between different receptors or isoforms may exist. Future studies are needed to address these issues.

In summary, we showed that there was a differential expression of ERs in HGSC and CCC, high GPER-1 expression was an independent prognostic factor for PFS in patients with HGSC, and GPER-1 might play a role in the proliferation of HGSC cells. Further studies are needed to clarify the significance of GPER-1 expression in the survival of patients with ovarian cancer.

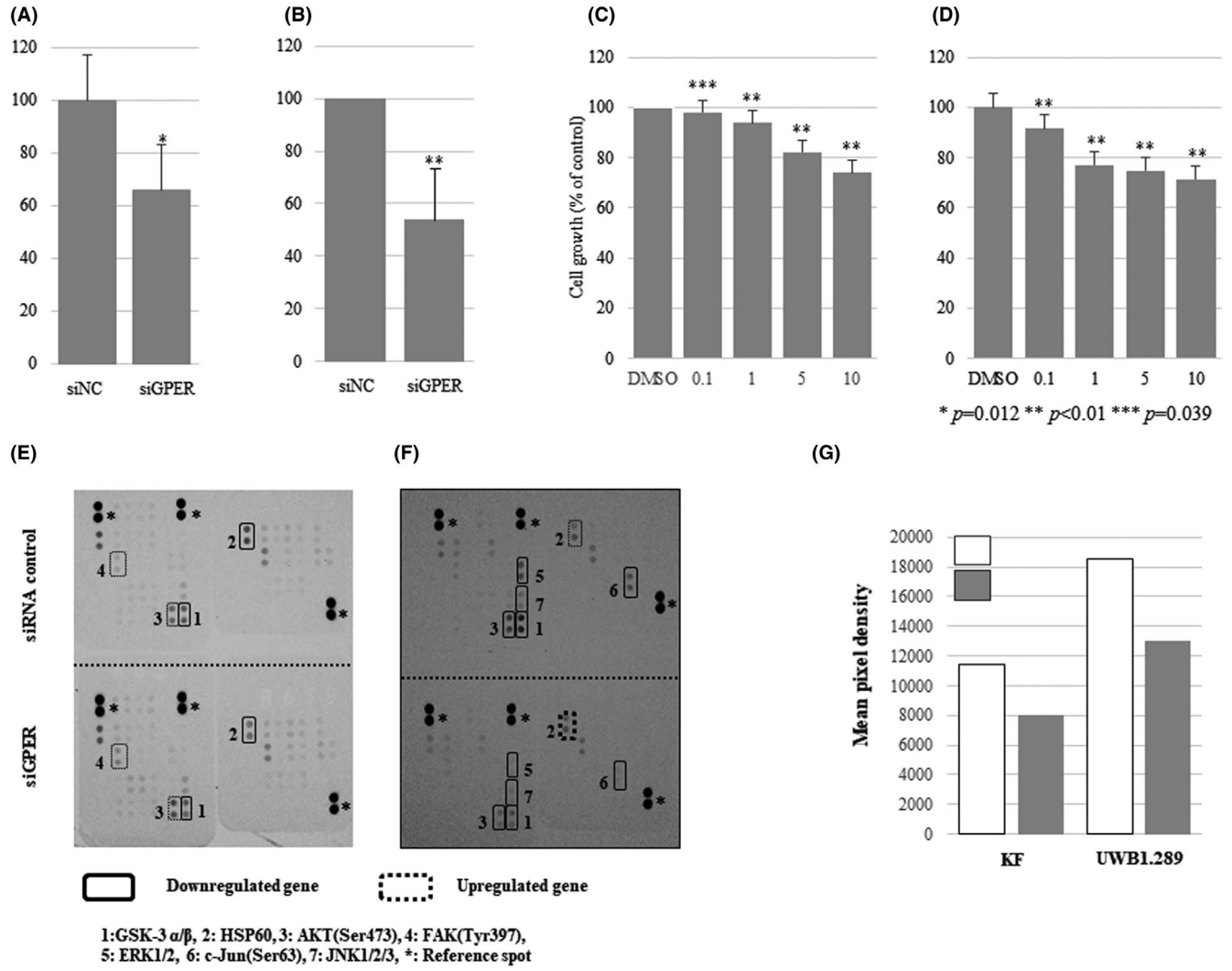

FIGURE 4 Effects of short interfering ribonucleic acid transfection specific for G protein-coupled estrogen receptor-1 on proliferation of (A) KF and (B) UWB1.289 cells. Effects of G-15 on proliferation of (C) KF and (D) UWB1.289 cells. Results are expressed as the percentage of the control value and presented as the mean ± standard error of at least six independent experiments. *$P = .012$, **$P < .01$, ***$P = .039$ versus the control. Representative autoradiography of the phosphokinase array for (E) KF and (F) UWB1.289. (G) Phospho-glycogen synthase kinase-3α/β expression after G protein-coupled estrogen receptor-1 knockdown. 1: glycogen synthase kinase-3 α/β; 2: heat shock protein 60; 3: protein kinase B (Ser473); 4: focal adhesion kinase (Tyr397); 5: extracellular signal-regulated kinase 1/2; 6: c-Jun (Ser 63); 7: c-Jun N-terminal kinases 1/2/3; *: Reference spot

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the study's conception and design. Daiken Osaku, Naoshi Kawamura, and Tetsuro Oishi contributed to material preparation and data collection and analysis. Daiken Osaku contributed to the first draft of the manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL

The present study was approved by the Institutional Review Board of Tottori University Hospital (IRB number 19-A198).

HUMAN RIGHTS STAREMENTS AND INFORMED CONSENT

Written informed consent was obtained from all patients according to the institutional guidelines.

ORCID

Tetsuro Oishi https://orcid.org/0000-0001-9374-0101

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012: estimated cancer incidence, mortality and prevalence worldwide in 2012 v1.0. IARC Cancerbase No. 11. International Agency for Research on Cancer. Lyon, France. 2013. http://globocan.iarc.fr. (accessed 14 Jul 2016)
2. Cancer Information Service, National Cancer Center. Cancer registry and statistics (Vital statistics of Japan). Japan. https://ganjoho.jp/reg_stat/statistics/dl/index.html#mortality. (accessed 14 Apr 2021)
3. Sugiyama T, Kamura T, Kigawa J, et al. Clinical characteristics of ovarian clear cell carcinoma: a distinct histologic type with poor prognosis and resistance to platinum-based chemotherapy. *Cancer*. 2000;88(11):2584-2589.
4. Itamochi H, Kigawa J, Terakawa N. Mechanisms of chemoresistance and poor prognosis in ovarian clear cell carcinoma. *Cancer Sci*. 2008;99(4):653-658.
5. Kolkova Z, Casslén V, Henic E, et al. The G protein-coupled estrogen receptor1 (GPER/GPR30) does not predict survival in patients with ovarian cancer. *J Ovarian Res*. 2012;5:9.
6. Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. *J Biol Chem*. 2001;276(40):36869-36872.
7. Prossnitz ER, Barton M. The G-protein-coupled estrogen receptor GPER in health and disease. *Nat Rev Endocrinol*. 2011;7:715-726.
8. Sieh W, Köbel M, Longacre TA, et al. Hormone-receptor expression and ovarian cancer survival: an ovarian tumor tissue analysis consortium study. *Lancet Oncol*. 2013;14(9):853-862.
9. Gourley C. Hormone receptors and ovarian cancer survival. *Lancet Oncol*. 2013;14(9):794-795.
10. Izawa M, Taniguchi F, Harada T. Molecular background of estrogen receptor gene expression in endometriotic cells. *Reprod Sci*. 2016;23(7):871-876.
11. Owman C, Blay P, Nilsson C. Cloning of human cDNA encoding a novel heptahelix receptor expressed in Burkitt's lymphoma and widely distributed in brain and peripheral tissues. *Biochem Biophys Res Commun*. 1996;228(2):285-292.
12. Prossnitz ER, Oprea TI, Sklar LA, Arterburn JB. The ins and outs of GPR30: a transmembrane estrogen receptor. *J Steroid Biochem Mol Biol*. 2008;109(3-5):350-353.
13. Smith HO, Arias-Pulido H, Kuo DY, et al. GPR30 predicts poor survival for ovarian cancer. *Gynecol Oncol*. 2009;114:465-471.
14. Fujiwara S, Terai Y, Kawaguchi H, et al. GPR30 regulates the EGFR-Akt cascade and predicts lower survival in patients with ovarian cancer. *J Ovarian Res*. 2012;5:35.
15. Ignatov T, Modl S, Thulig M, et al. GPER-1 acts as a tumor suppressor in ovarian cancer. *J Ovarian Res*. 2013;6:51.
16. Schüler-Toprak S, Skrzypczak M, Ignatov T, Ignatov A, Ortmann O, Treeck O. G protein-coupled estrogen receptor 1 (GPER-1) and agonist G-1 inhibit growth of ovarian cancer cells by activation of anti-tumoral transcriptome responses: impact of GPER-1 mRNA on survival. *J Cancer Res Clin Oncol*. 2020;146:3175-3188.
17. Komatsu H, Oishi T, Itamochi H, et al. Serum vascular endothelial growth factor-A as a prognostic biomarker for epithelial ovarian cancer. *Int J Gynecol Cancer*. 2017;27(7):1325-1332.
18. Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue (in Germany). *Pathologe*. 1987;8:138-140.
19. Zhu CX, Xiong W, Wang ML, et al. Nuclear G protein-coupled oestrogen receptor (GPR30) predicts poor survival in patients with ovarian cancer. *J Int Med Res*. 2018;46(2):723-731.
20. Akahane T, Sekizawa A, Okuda T, Kushima M, Saito H, Okai T. Disappearance of steroid hormone dependency during malignant transformation of ovarian clear cell cancer. *Int J Gynecol Pathol*. 2005;24:369-376.
21. Chan KKL, Siu MKY, Jiang YX, et al. Differential expression of estrogen receptor subtypes and variants in ovarian cancer: effects on cell invasion, proliferation and prognosis. *BMC Cancer*. 2017;17:606.
22. Ciucci A, Zannoni GF, Travaglia D, Petrillo M, Scambia G, Gallo D. Prognostic significance of the estrogen receptor beta (ERβ) isoforms ERβ1, ERβ2, and ERβ5 in advanced serous ovarian cancer. *Gynecol Oncol*. 2014;132(2):351-359.
23. Bang D, Wilson W, Ryan M, Yeh JJ, Baldwin AS. GSK-3α promotes oncogenic KRAS function in pancreatic cancer via TAK1-TAB stabilization and regulation of non-canonical NF-κB. *Cancer Discov*. 2013;3:690-703.
24. Cao Q, Lu X, Feng YJ. Glycogen synthase kinase-3beta positively regulates the proliferation of human ovarian cancer cells. *Cell Res*. 2006;16:671-677.
25. Sun A, Shanmugam I, Song J, Terranova PF, Thrasher JB, Li B. Lithium suppresses cell proliferation by interrupting E2F-DNA interaction and subsequently reducing S-phase gene expression in prostate cancer. *Prostate*. 2007;67(9):976-988.

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