LRH1 promotes tumor cell proliferation, migration and correlates with poor prognosis in ovarian cancer

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Abstract

Background Recent studies have revealed that liver receptor homolog 1 (LRH1) plays a vital role in several human cancers, but its role in ovarian cancer (OC) remains unclear. We aimed to investigate whether LRH1 protein can act as a promising biomarker to predict the prognosis and metastasis in OC patients.

Methods LRH1 expression status was evaluated by immunohistochemical and reverse transcription (RT)-qPCR analysis. The effects of LRH1 on tumor cell proliferation and progression were evaluated in vitro.

Results RT-qPCR showed that LRH1 mRNA expression was more intense in the invasive lesions ($P < 0.05$). LRH1 overexpression were extremely related with elevated FIGO stage ($P = 0.001$), lymph node metastasis ($P = 0.011$), and peritoneal metastasis ($P = 0.001$). Furthermore, LRH1 expression was an independently prognostic index for disease-free survival in OC patients ($P = 0.041$). In addition, LRH1 overexpression ($P = 0.011$) along with FIGO stage ($P < 0.001$) and ascites ($P = 0.015$) independently affect peritoneal metastasis in OC patients. LRH1 knockdown significantly inhibited the proliferation, migration, and metastasis of human OC cells ($P < 0.05$).

Conclusions The present study provides evidence that LRH1 predicts peritoneal metastasis and poor outcome, highlighting its potential role as biomarker for targeted therapy in OC.

Background

The incidence of ovarian cancer (OC) has showed a steady decrease during the past ten years in American, however its mortality rate remains high [1, 2]. Surgery is the priority and standard treatment for OC. Recent years, even though novel technology has been applied for the diagnosis and treatment of OC, while the prognosis remain poor for advanced stage of OC [3]. We have gained insight on ovarian cancer in recent years although advancing knowledge has previously been hindered by substantial disease heterogeneity and uncertainties about tumor tissues of origin [2]. Therefore, it will be helpful to identify an ideal biological factor which predicts the malignant biological behavior and the prognosis of OC.

LRH1 (also known as nuclear receptor subfamily 5 group A member 2 NR5A2) participates in various biological processes: liver and pancreas differentiation, steroidogenesis in childhood, cholesterol/bile acid homeostasis, and tumor progression [4, 5]. Previous studies have validated that overexpression of LRH1 may be induced resistance to chemotherapy of breast cancer [6], metastasis of pancreases cancer [7], poor prognosis of colon cancer [8] and non-small cell lung cancer [9], and proliferation of hepatoblastoma [10]. However, LRH1 expression in OC patients has not been reported so far.

Thus, we aimed to explore the status of LRH1 protein expression in OC and its clinical significance.

Materials And Methods

Human tissue samples and clinical data

133 OC tumor tissue samples were obtained from Harbin Medical University Cancer Hospital, who underwent cytoreductive surgery without any prior treatment for cancer such as chemotherapy or radiation between January 2009 and December 2011. The International Federation of Gynecology and Obstetrics (FIGO) staging system was applied to assess tumor stage [11]. Histological grades were appraised on the basis of the standard of the World Health Organization Histological Grading System for tumors [12]. Furthermore, to assess the status of lymph node, all included OC patients underwent lymph node dissection. Besides, 25 patients with normal ovaries who underwent hysterectomy with oophorectomy for benign uterine disease in our hospital during the same period were included.

Approval from the Medical Ethics Committee of Harbin Medical University Cancer Hospital was obtained for the purpose of research. 133 OC patients were followed up for survival analyses until death or until the study closing date (January 2018).

Immunohistochemical (IHC) staining and evaluation

133 tissue blocks of OC and 25 of normal ovary tissues were cut by a microtome into 4- μ m sections and affixed onto the slide. After dewaxing in xylene and rehydrating through graded alcohol concentrations, the sections were incubated with 0.3% hydrogen peroxide for 10 minutes at room temperature to block endogenous peroxidase, and then the sections were incubated with anti-LRH1 antibody (1:100, Abcam, ab223211) overnight at 4°C. After washing in phosphate-buffered saline (PBS), all sections were incubated with secondary antibodies (R&D Systems, NL004) at room temperature for 20 min and then treated with 3,3'-diaminobenzidine tetrahydrochloride (Dako, Hamburg, Germany), following by counterstaining with hematoxylin.

Protein expression level of LRH1 was scored by evaluating the percentage of the positive staining areas of tumor cells together with intensity of staining. The former was scored as follows [9]: 0, < 0%; 1, 1–10%; 2, 11–50%; 3, 51–70% and 4, $\geq$ 70%. The latter was scored as follows: 0, negative staining; 1, weak staining; 2, moderate staining; and 3, intense staining. The final expression level of LRH1 was semi-quantitatively evaluated according to the sum of the scores for the percentage of positively stained tumors cells and intensity scores (0–7) in which the final staining scores of 0–3 and 4–7 were considered to be low and high expression, respectively.

The IHC staining on each slide was scored in twice independently by two pathologists who were sophisticated in evaluating IHC and blinded with the clinicopathological information.

Cell culture

The human OC cell lines SKOV3 and OVCAR3 (ATCC) were employed. Both the cell lines have been approved by short tandem repeat (STR) profiling. Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO₂ in DMEM complemented with 10% FBS and antibiotics (penicillin and streptomycin). Cells were passaged when they reached 80% confluence.

Western blotting analysis

The cell lysates were both centrifuged at 13000 rpm (at 4°C for 5 minutes). After that 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) were used to segregate them, and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The filters were blocked with blocking buffer (Sangon Biotech) for 45 min. Membranes were incubated with Primary antibodies against LRH1 (Abcam, ab223211) at 4°C overnight, and with secondary antibodies at 1:5000 dilution at room temperature for 2 h, following by incubating with β -actin (Abcam, ab179467) at room temperature for 1.5 h.

RNA extraction and reverse transcription (RT)-qPCR

The TRIzol™ LS Reagent (Invitrogen™) was used to extract total RNA from tissues and cells, according to the manufacturer's protocol, followed by complementary DNA synthesis using a PrimeScript RT reagent Kit with gDNA Eraser (Takara). Amplification was subsequently carried out using the GeneAmp® PCR System 9700 (Applied Biosystems, USA). Real-time PCR was performed using LightCycler® 480 Real-time PCR Instrument (Roche, Swiss). The mRNA expression levels of LRH-1 were normalized to the mRNA levels of GAPDH, which was used as an internal control. The $2^{-\Delta\Delta C_t}$ method was used to quantify the mRNA expression levels [13].

Establishment of stable short hairpin (sh)RNA-mediated LRH-1 knockdown ovarian cancer cell lines

shRNA-induced knockdown of LRH-1 expression was achieved using the lentiviral expression system. The plasmid used in the present study were pPLK/GFP + Puro-NR5A2 (Genomeditech, China). Viral particles were generated by co-transfecting 293T cells (ATCC) with the shRNAs and the GM easy™ Lentivirus Packaging kit (Genomeditech, China), which contains packaging plasmids and a transfection reagent according to the manufacturer's protocol. Subsequently, the shRNA viral particles transfected SKOV3 and OVCAR3 cells with 4 μ g/mL polybrene (Sigma-Aldrich), and stable cell lines were established after 10 days of puromycin (2 μ g/ml) selection. Knockdown was confirmed using RT-qPCR or immunoblotting. The selected cell lines were routinely cultured in puromycin-containing media until 2 days prior to experimentation.

Transwell assay

For migration assays, infected OVCAR3 cells (1×10^5 in 200 μ L of serum-free DMEM medium) and SKOV3 cells (1×10^5 in 200 μ L of serum-free DMEM medium) were seeded into the upper chamber of transwell plates in a 24-well format with 8 μ m diameters (Corning Costar, USA). Then, 600 μ L of medium containing 10% FBS was added to the bottom chamber as a chemoattractant. After 24 hours of culture, cells were fixed with methanol and stained with Crystal Violet solution. The remaining cells were removed from the top of the permeable membrane using a cotton swab. Then, cells that migrated through the

upper chamber were counted in 4 random fields under a light microscope (NIKON INSTRUMENTS(SHANGHAI)CO.,LTD).

Wound healing assay

OVCAR3 (1×10^5 cells) and SKOV3 cells (1×10^6 cells) were seeded in 6-well cell culture plates and incubated for 24 hours at 37°C. After achieving confluence, the cellular layer in each plate was scratched using a plastic pipette tip. The migration of the cells at the edge of the scratch was analyzed at 0 and 24 hours, when microscopic images of the cells were captured.

Cell counting kit-8 (CCK-8)

SKOV3 and OVCAR3 cells were plated in flat-bottom 96-well plates (1500 and 1000 cells/well) and supplemented with 100μL DMEM medium with 10%FBS per well. After incubation at 37 °C in a humidified incubator with 5% CO₂ for 2, 24, 48, 72, and 96 hours, respectively, 10 μL of CCK-8 (Yeasen Biotech Co., Ltd.) was added to each well. Then, after 1 hours of culture, colorimetric analysis was performed on a microplate reader (Thermo Scientific™ Varioskan™ LUX) at a wavelength of 450 nm. The assay was performed using six replicates.

Statistical analysis

The chi-square test was used to analyze the statistical differences of the clinicopathologic variables. Overall survival (OS) and disease-free survival (DFS) were evaluated by the Kaplan-Meier method and log-rank test. The Cox proportional hazards model was used to estimate the independent prognostic factors for survival. Logistic regression was performed for multivariate analysis of the association between LRH1 expression and intraperitoneal metastasis. Student's t-test were used to analyze the differences between the experimental and control groups. A two-sided $P < 0.05$ was considered significant.

Results

Expression level of LRH1 in OC tissues

RT-qPCR was carried out to identify the discrepancy between LRH1 mRNA expression in OC and normal ovarian tissues (**Figure. 1**). No discrepancy was observed between LRH1 expression in normal tissues and that in OC tissues ($P > 0.05$). However, LRH1 protein in OC with metastasis presents (T2) higher expression than that in OC without metastasis (T1) ($P = 0.017$). Moreover, the status of LRH1 mRNA expression was more intense in the invasive foci (M) than in the situ foci (T2) among the same patients ($P = 0.034$).

IHC revealed that the expression of LRH1 in normal tissues were scarcely detectable, however, LRH1 in OC tissues was stained intensely (**Figure. 2**). As shown in Fig. 2B, LRH1 was observed both in both the nucleus and cytoplasm of tumor cells. However, LRH1 was mainly localized in the nucleus of tumor cells. Of the OC specimens examined, low LRH1 expression was detected in 59 of 133 (44.4%) patients, and high LRH1 expression was detected in 74 of 133 (55.6%) patients.

Relationship of elevated LRH1 protein expression with clinicopathologic variables

Table 1 summarizes the association between LRH1 expression and clinicopathological variables in OCs. LRH1 expression significantly related with the FIGO stage ($P= 0.001$), lymph node metastasis ($P= 0.011$), and intraperitoneal metastasis ($P= 0.001$). No relationships of LRH1 with age, histological type, histologic grade, residual disease, ascites, or serum CA-125 level were observed ($P> 0.05$).

Table 1
Correlation between LRH1 protein expression level and clinicopathological variables in 133 patients with OC

Variables	All patients n = 133	LRH1 expression level		<i>P</i>
		Low (%) (n = 59)	High (%) (n = 74)	
Age (years)	67	33 (49.3)	34 (50.7)	0.252
≤ 52	66	26 (39.4)	40 (60.6)	0.653
> 52	92	42 (45.7)	50 (54.3)	0.001
Histological type	41	17 (41.5)	24 (58.5)	0.310
Serous	28	20 (71.4)	8 (28.6)	0.851
Others	105	39 (37.1)	66 (62.9)	0.473
FIGO stage	50	25 (50.0)	25 (50.0)	0.653
I/II	83	34 (41.0)	49 (59.0)	0.011
III/IV	98	43 (43.9)	55 (56.1)	0.001
Histologic grade	35	16 (45.7)	19 (54.3)	
G1/G2	38	15 (39.5)	23 (60.5)	
G3	95	44 (46.3)	51 (53.7)	
Residual disease (cm)	41	17 (41.5)	24 (58.5)	
< 1	92	42 (45.7)	50 (54.3)	
≥ 1	81	43 (53.1)	38 (46.9)	
Ascites (ml)	52	16 (30.8)	36 (69.2)	
< 100	43	28 (65.1)	15 (34.9)	
≥ 100	90	31 (34.4)	59 (65.6)	
Serum CA-125 level (U/ml)				
≤ 35				
> 35				
Lymph node metastasis				
No				
Yes				
FIGO the Federation of Gynaecology and Obstetrics, G1 Well differentiated, G2 moderately differentiated, G3 poorly differentiated, OC ovarian cancer				

Variables	All patients n = 133	LRH1 expression level		<i>P</i>
		Low (%)	High (%)	
		(n = 59)	(n = 74)	
Peritoneum metastasis				
No				
Yes				
FIGO the Federation of Gynaecology and Obstetrics, G1 Well differentiated, G2 moderately differentiated, G3 poorly differentiated, OC ovarian cancer				

LRH1 increased expression in OC predicts an unfavorable prognosis especially recurrence

The log-rank test showed that patients with low LRH1 expression had a significantly better OS and DFS compared to patients with high LRH1 expression ($P= 0.007$ and $P< 0.001$, Fig. 3). In addition to LRH1, FIGO stage (both $P< 0.001$), histologic grade ($P= 0.009$ and $P= 0.028$, respectively), residual disease $P= 0.022$ and $P= 0.026$, respectively), lymph node metastasis (both $P< 0.001$), and intraperitoneal metastasis ($P= 0.001$ and $P< 0.001$, respectively) are correlated with prognosis of OC (Table 2). Furthermore, displayed in Table 2, multivariate Cox regression analysis showed that the high LRH1 expression was considered as an independent prognostic marker for DFS of patients with OC instead of OS ($P= 0.041$).

Table 2
Univariate and multivariate analyses for survival in 133 OC patients

Variables	Univariate	Multivariate	
	<i>P</i>	HR 95%CI	<i>P</i>
variables related to OS	0.219	3.695 (1.793–7.613)	< 0.001
Age (years)	0.914	1.664 (1.101–2.513)	0.016
Histological type	< 0.001	1.682 (1.093–2.591)	0.018
FIGO stage	0.009	3.665 (2.344–5.730)	< 0.001
Histologic grade	0.022	3.437 (1.641–7.200)	0.001
Residual disease (cm)	0.696	1.550 (1.024–2.345)	0.038
Ascites (ml)	0.154	1.663 (1.065–2.597)	0.025
Serum CA-125 level (U/ml)	< 0.001	3.857 (2.431–6.120)	< 0.001
Lymph node metastasis	0.001	1.535 (1.017–2.318)	0.041
Peritoneum metastasis	0.007		
LRH1 expression level	0.487		
variables related to DFS	0.895		
Age (years)	< 0.001		
Histological type	0.028		
FIGO stage	0.026		
Histologic grade	0.855		
Residual disease (cm)	0.281		
Ascites (ml)	< 0.001		
Serum CA-125 level (U/ml)	< 0.001		
Lymph node metastasis	< 0.001		
Peritoneum metastasis			
LRH1 expression level			
FIGO the Federation of Gynaecology and Obstetrics, OS overall survival, DFS disease-free survival, OC ovarian cancer, HR hazard ratio, CI confidence interval			

LRH1 increased expression was an indicator for intraperitoneal and lymph node metastasis in OC

To evaluate the relationship between LRH1 overexpression and intraperitoneal metastasis, traditional clinicopathological parameters about intraperitoneal metastasis were first appraised (Table 3). Univariate analyses of clinicopathologic variables for the metastasis of the intraperitoneal showed that the presence of intraperitoneal metastasis was positively associated with FIGO stage ($P < 0.001$), residual disease ($P = 0.025$), and ascites ($P = 0.019$). Multivariate logistic regression analysis revealed that FIGO stage ($P = 0.003$), residual disease ($P = 0.032$), and LRH1 ($P = 0.010$) were independently linked with intraperitoneal metastasis.

Table 3

Univariate and multivariable analyses for the relationship between LRH1 expression and lymph node metastasis in OCs

Variables	No.	Lymph node metastasis		P	OR (95%CI)	P
		Negative	Positive			
Age (years)	40	27		0.775	2.421 (1.109–5.285)	0.026
≤ 52	41	25		0.991		0.019
> 52	56	36		0.016	2.451 (1.162–5.173)	
Histological type	25	16		0.595		
Serous	37	13		0.653		
Others	44	39		0.991		
Histologic grade	61	37		0.011		
G1/G2	20	15				
G3	22	16				
Residual disease (cm)	59	36				
< 1	25	16				
≥ 1	56	36				
Ascites (ml)	43	16				
< 100	38	36				
≥ 100						
Serum CA-125 level (U/ml)						
≤ 35						
> 35						
LRH1 expression level						
Low						
High						
OC ovarian cancer, HR hazard ratio, CI confidence interval						

Using the same method, the results showed that the presence of lymph node metastasis was significantly correlated with the histologic grade ($P = 0.016$) and LRH1 expression ($P = 0.011$). Multivariate logistic

regression that histologic grade ($P= 0.026$) and LRH1 expression ($P= 0.019$) significantly led to lymph node metastasis (Table 4).

Table 4

Univariate and multivariable analyses for the relationship between LRH1 expression and peritoneum metastasis in OCs

Variables	No.	Peritoneum metastasis		P	OR (95%CI)	P
		Negative	Positive			

Variables	No.	Peritoneum metastasis		P	OR (95%CI)	P
		Negative	Positive			
Age (years)	22	45	0.900	4.613 (1.678–12.677) 3.208 (1.104–9.322) 3.160 (1.323–7.545)	0.003	
≤ 52	21	45	0.918		0.032	
> 52	30	62	< 0.001		0.010	
Histological type	13	28	0.064			
Serous	19	9	0.025			
Others	24	81	0.019			
FIGO stage	21	29	0.614			
I/II	22	61	0.001			
III/IV	37	61				
Histologic grade	6	29				
G1/G2	18	20				
G3	25	70				
Residual disease (cm)	12	29				
< 1	31	61				
≥ 1	28	31				
Ascites (ml)	15	59				
< 100						
≥ 100						
Serum CA-125 level (U/ml)						
≤ 35						
> 35						
LRH1 expression level						
Low						
High						
FIGO the Federation of Gynaecology and Obstetrics, OC ovarian cancer, HR hazard ratio, CI confidence interval						

Knockdown of LRH1 inhibits OC cells proliferation and migration in vitro

LRH1 knockdown assays using sh-RNA was conducted to investigate the biological function of LRH1 in OC cells. We used RT-qPCR and Western blotting to confirm that LRH1 expression in OVCAR3 and SKOV3 with sh-LRH1 transfection was significantly lower than that with sh-NC transfection (Fig. 4A and B). We explored the effect of knockdown of LRH1 on cell proliferation in OVCAR3 and SKOV3 cells. Compared with OVCAR3 and SKOV3 cells infected with sh-NC, the proliferation of OVCAR3 and SKOV3 cells infected with sh-LRH1 was repressed at 24, 48, 72, and 96 hours, respectively (Fig. 4C). Thus, knockdown of LRH1 significantly repressed cell proliferation.

We also investigate the effect of LRH1 on migration. Migrated cells through the transwell plates were counted in three random fields, and the average value of the three fields was calculated. The result of transwell assay showed that knockdown of LRH1 decreased the migration of SKOV3 and OVCAR3 cells by approximately 43% and 50%, respectively (Fig. 4D). Wound-healing assay showed that LRH1 promoted cell migration in the SKOV3 and OVCAR3 cells (Fig. 4E). After 24 h, SKOV3 and OVCAR3 control cells migrated approximately 20 mm and 12 mm, respectively and SKOV3 and OVCAR3 with LRH1 knockdown migrated approximately 13 mm and 8 mm, respectively. Cells with LRH1 knockdown shows less motility than control cells, indicating that LRH1 could stimulate cell migration.

Discussion

The new diagnosed cases and deaths of ovarian cancer will respectively be 22,240

and 14,070 in the United States. OC involves a heterogenous group of malignancies that differ from etiology, molecular biology, and other characteristics [2]. OC is the most common malignant tumor. High-risk ovarian carcinoma patients have very low cure rates, despite therapy comprised of combined treatments, including surgery, chemotherapy and radiation [2]. Clearly, identification of novel biomarkers and more effective therapies is extremely urgent.

LRH1 is a member of the nuclear receptor NR5A (Ftz-F1) subfamily which expresses in endodermal origin tissues containing the intestine, liver, exocrine pancreas [14], ovary [15–18], pre-adipocyte [19], and placenta [20]. Recent studies have reported that LRH1 protein functioned as a cancer regulator which exist in extensive procedures, such as cell proliferation, chemotherapy resistance, and tumor progress [6, 21, 22]. In the present study, we noticed that LRH1 protein expression showed high expression in OC tissues. Moreover, it indicated an increased trend of LRH1 protein and mRNA expression from T1 to T2 and its matched M. This study firstly identified overexpression of LRH1 in OC tissues. Preceding studies showed similar result in non-small cell lung cancer tissues and colon cancer tissues. Liu et al. [9] demonstrated that LRH1 was highly overexpressed in non-small cell lung cancer tissues compared with normal tissues. Wu et al. [8] carried out IHC in colon cancer tissues to test LRH1 expression, which indicated that comparing with the adjacent normal tissues, the protein level of LRH1 was outstandingly

elevated in the cancerous tissues. Additionally, Jin et al. [10] verified LRH1 expression in three hepatoblastoma cell lines, HepG2, HuH6 and HepT1, and one control cell line, THLE-2 by western blotting and found that LRH1 expression was also remarkably upgraded in HepG2 cells and HuH6 cells. Thus, LRH1 may provide a candidate molecular marker for malignant behavior of tumor.

A number of studies have viewed LRH1 as a significant prognosis biomarker for tumor invasion and proliferation in several carcinomas, for example pancreases cancer [23]. Enlightened by these researches, we were prompted to identify the role of LRH1 overexpression as a predictor for clinicopathological and prognostic significance. We recognized that elevated LRH1 protein expression was tremendously corresponded with FIGO stages, lymph node metastasis and intraperitoneal metastasis. Furthermore, multivariate logistic regression model demonstrated that high standard of LRH1 expression was significantly associated with lymph node metastasis and intraperitoneal metastasis. In addition, the OS periods in patients with high LRH1 expression were shorter than that in patients with low LRH1 expression. Oppositely, patients whose LRH1 expression is low presented better prognoses than those with high LRH1 expression. Wang et al. [24] adopted bioinformatics analysis to screen on the focus of the nuclear receptor (NR) family. They found that overexpression of these orphan NRs could increase cancer stem cell markers expression and enhanced spheroid formation capacity in prostate cancer cells. Moreover, they concluded that LRH1 could be the potential therapeutic targets for PCSCs or castration-resistant prostate cancer.

The mechanism of LRH1 influencing tumorigenesis, cell proliferation, migration, chemotherapy resistance varies in different kinds of cancers. Our study showed that LRH1 down-regulation inhibited OC cells proliferation and migration in vitro. In pancreases cancer, Lin et al. [7] have demonstrated that LRH1 enhanced transcriptional activity of β -catenin and up-regulated the expression of downstream target genes (c-Myc, MMP2/9), thus promoted migration and invasion. In breast cancer, it has found that MDC1 transcription was promoted by LRH1 through directly activating MDC1 promoter and resulting in elevated γ H2AX levels. Furthermore, the mRNA level of LRH1 and MDC1 were positively correlated in recurrent breast cancer samples. In addition, LRH1 promoted breast cancer cell chemoresistance by enhancing the expression of MDC1 and attenuating DNA damage [6]. All of these findings suggest an important biological role of LRH1 in carcinogenesis and tumor progression.

Conclusion

Although limitations still exist in this study, the present results we observed showed that LRH1 contributes to the lymph node metastasis, intraperitoneal metastasis and poor prognosis in OC, which indicated that LRH1 could be a specific biomarker for predicting poor prognosis and metastasis.

Abbreviations

LRH1: liver receptor homolog 1; OC: ovarian cancer; RT: reverse transcription; FIGO: the International Federation of Gynecology and Obstetrics; PBS: phosphate-buffered saline; IHC: Immunohistochemical;

STR: short tandem repeat; SDS–PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF: polyvinylidene difluoride; OS: Overall survival; DFS: disease-free survival

Declarations

Authors' contributions:

YLB conceived and designed the study. LJX and LJM collected samples and processed data. SQT provided technical support. SWZ and YLB analyzed data. SWZ drafted the manuscript. YLB revised the manuscript. All authors read and approved the final manuscript.

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Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

All data generated and analyzed during this study are included in this published article and its additional file.

Consent for publication:

Not applicable.

Ethics approval and consent to participate:

The current study was approved by the Ethics Committee of Harbin Medical University Cancer Hospital. A written consent from each participant was obtained after they were informed the purpose of this study.

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Figures

Figure 1

Real time PCR assay of LRH1 mRNA expression in N, T1, T2, and its matched M. It showed that elevated trend of LRH1 mRNA expression from N to T1, T2 and its matched M (P < 0.05).

Figure 2

Immunohistochemical anlaysis of LRH1 protein expression (x 400). A negative or weak expression in normal ovarian tissues; B high expression in primary ovarian cancer (OC) tissues (observed mainly in

nucleus, a few in cytoplasmic); C low expression in serous histotypes (mainly in nucleus); D high expression in serous histotypes (mainly in nucleus, a few in cytoplasmic); E high expression in mucinous histotypes (mainly in nucleus); F high expression in endometrioid histotypes (mainly in nucleus, a few in cytoplasmic); G negative or low expression in clear cell histotypes; H high expression in clear cell histotypes (mainly in nucleus, scarcely in cytoplasmic).

Figure 3

Kaplan–Meier analysis of overall survival (OS) and disease-free survival (DFS) related to the expression of LRH1. A OS curves of the 133 OC patients; B DFS curves of the 133 OC patients.

Figure 4

Knockdown of LRH1 inhibits ovarian cancer cells proliferation and migration in vitro. The level of mRNA (A) and protein (B) expression significantly decreased after sh-LRH1 transfection. CCK8 assays (C) have revealed that knockdown of LRH1 inhibits cell proliferation. Transwell assays (D) and wound healing assays (E) have indicated that knockdown of LRH1 inhibits cell migration. **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$; data are shown as the mean $\pm$ SD.