Gene microarray analysis of lncRNA and mRNA expression profiles in patients with high-grade ovarian serous cancer

YANHUI LOU, HUANHUAN JIANG, ZHUMEI CUI, XIANGYU WANG, LINGZHI WANG and YI HAN

Department of Gynecology, Affiliated Hospital of Qingdao University, Qingdao, Shandong 266100, P.R. China

Abstract. High-grade ovarian serous cancer is known for its high rates of invasion and metastasis, and resultant high mortality rate. Therefore, research concerning biomarkers and underlying molecular mechanisms of high-grade ovarian serous cancer progression and prognosis are urgently required. Long non-coding RNAs (lncRNAs) have been the subject of an increasing number of studies, and certain lncRNAs have been demonstrated to serve an important function in the development and progression of various cancers, including HOX transcript antisense RNA, competing endogenous lncRNA 2 for microRNA let-7b, urothelial cancer associated 1, and H19, imprinted maternally expressed transcript (non-protein coding). However, few studies have investigated the differential expression of lncRNAs in high-grade ovarian serous cancer. In the present study, differences in lncRNA and mRNA expression profiles between high-grade ovarian serous cancer tissue samples and healthy fallopian tube tissue samples were investigated using microarray analysis, and the differential expression of lncRNAs and mRNAs was confirmed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Then, five abnormally expressed lncRNAs were selected, and the associations between these lncRNAs and ovarian cancer clinicopathological parameters were examined using RT-qPCR. The expression profiles of certain lncRNAs and mRNAs were confirmed to be altered between high-grade ovarian serous cancer tissues and healthy fallopian tube tissues. Furthermore, the expression levels of selected lncRNAs were associated with International Federation of Gynecology and Obstetrics stage and lymph node metastasis. These lncRNAs and mRNAs may therefore be involved in the pathogenesis of high-grade ovarian serous cancer. The results of the present study provide an experimental foundation for further exploration of the value of these lncRNAs and mRNAs in the early diagnosis and treatment of high-grade ovarian serous cancer.

Introduction

Ovarian cancer has the highest mortality rate of all gynecologic malignancies (1). The disease is split into a number of classifications, including epithelial ovarian cancer, which accounts for 85-90% of malignant ovarian tumors (1). High-grade ovarian serous cancer is an aggressive type of epithelial ovarian cancer, with a high degree of malignancy and rapid progression. Compared with early-stage, high-grade ovarian serous cancer, the prognosis of patients with advanced high-grade ovarian serous cancer is poor, and the 5-year survival rate for these patients is only 27% (2). Adjacent organ invasion, multiple peritoneal implantation metastasis and lymph node metastasis are also observed in the majority of patients with advanced high-grade ovarian serous cancer (3). Therefore, studies investigating high-grade ovarian serous cancer development, progression-associated genes and regulatory mechanisms are necessary. Through these studies, researchers hope to improve our understanding of high-grade ovarian serous cancer progression and development, and identify long-term and effective treatment strategies for this disease.

The development of biological chips and high-throughput sequencing technology has revealed that it is possible to transcribe ~70-90% of the human genome to RNAs, but only 1-2% of RNAs are ultimately translated to proteins (4,5). The vast majority of RNAs do not have a protein-encoding function. These RNAs were classified as 'non-coding RNAs', and were originally thought to be either noise or waste products of transcription. With further research, non-coding RNAs were demonstrated to participate in a variety of physiologic and pathologic processes and to serve an important function in cancer progression (6). Long non-coding RNAs (lncRNAs) are a type of non-coding RNA, transcribed from intergenic and intronic regions in the human genome by RNA polymerase II, ranging in length from 200 nt to 100 kb and lacking significant protein-coding open reading frames (7). The reported biological functions of lncRNAs include DNA damage repair, epigenetic control, transcription regulation, pre- and post-translational regulation, control of the cell cycle, survival, migration, metabolism and differentiation, and even control

Correspondence to: Dr Yanhui Lou, Department of Gynecology, Affiliated Hospital of Qingdao University, 59 Haier Road, Qingdao, Shandong 266100, P.R. China
E-mail: lyh7497@163.com

Abbreviations: lncRNA, long non-coding RNA

of the apoptotic process (8-11). Furthermore, a number of lines of evidence link the dysregulation of lncRNAs to diverse human diseases, particularly cancer (12-16). The lncRNA research field is, therefore, very promising. However, studies concerning lncRNAs and their function and regulation mechanisms remain in their infancy, particularly those concerning high-grade ovarian serous cancer. At present, few lncRNAs have been reported as associated with high-grade ovarian serous cancer, including HOX transcript antisense RNA (*HOTAIR*), HOXA11 antisense RNA, competing endogenous lncRNA 2 for microRNA let-7b, maternally expressed 3 and urothelial cancer associated 1 (*UCA1*) (17-21). Furthermore, the differential expression profiles of lncRNAs between high-grade ovarian serous cancer and healthy fallopian tube tissues, and their functional significance, remain unclear. The aim of the present study was to explore the expression profiles of lncRNAs and their potential target genes in high-grade ovarian serous cancer tissues, and to analyze the associations between these lncRNAs and clinicopathological features of ovarian cancer. The results of the present study may represent evidence supporting the involvement of lncRNA expression levels in the progression of high-grade ovarian serous cancer, and establish a foundation for the development of potential diagnostic biomarkers and therapeutic targets for the treatment of high-grade ovarian serous cancer.

Materials and methods

Patients and tissue specimens. High-grade ovarian serous cancer tissue specimens were obtained from patients (average age, 55 years) who had undergone surgical treatment for ovarian cancer at the Affiliated Hospital of Qingdao University (Qingdao, China) between March 2015 and March 2016. Healthy fallopian tube tissue specimens were obtained from patients (average age, 53 years) who had undergone surgical treatment for hysteromyoma at the Affiliated Hospital of Qingdao University during the same period. All cases were confirmed by postoperative pathological diagnosis. Patients who had received neoadjuvant chemotherapy or radiation therapy prior to surgery were excluded from the present study. A total of 23 high-grade ovarian serous cancer tissue samples and 23 healthy fallopian tube tissue samples were collected. Of these samples, 3 high-grade ovarian serous cancer samples (labeled A1-3) and 3 healthy fallopian tube tissue samples (labeled B1-3) were used for global profiling of human lncRNA and mRNA expression using the Arraystar human lncRNA microarray (Arraystar, Inc., Rockville, MD, USA). A further 20 pairs of samples were used for confirmation of differential lncRNA and mRNA expression by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. In addition, 40 high-grade ovarian serous cancer tissue samples were collected to analyze the associations between differential lncRNA expression and ovarian cancer clinicopathological parameters, using RT-qPCR analysis. These samples were obtained from patients (average age, 55 years) who had undergone surgical treatment for ovarian cancer at the Affiliated Hospital of Qingdao University between March 2016 and June 2017. All samples were stored at -80˚C immediately following surgical resection. All subjects provided signed statements of written informed consent, and all experimental procedures were approved by the Institutional Review Board of the Ethics Board of the Affiliated Hospital of Qingdao University.

RNA extraction and quality control. Total RNA was extracted from the tissue samples using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol. The entire process was performed on ice. The quantity and quality of the RNA samples were measured using a NanoDrop ND-1000 spectrophotometer. RNA integrity was assessed using standard 1% denaturing agarose gel electrophoresis. Isolated RNA was stored at -80˚C and prepared for lncRNA array analysis and RT-qPCR.

RNA labeling and array hybridization. Sample labeling and array hybridization were performed according to the Agilent one-color microarray-based gene expression analysis protocol (Agilent Technologies, Inc., Santa Clara, CA, USA) with minor modifications. Briefly, mRNA was purified from the total RNA following removal of rRNA (using an mRNA-ONLY™ Eukaryotic mRNA Isolation kit; Epicentre; Illumina, Inc., San Diego, CA, USA). Then, each sample was amplified and transcribed into fluorescent cRNA along the entire length of the transcripts without 3' bias, utilizing a random priming method (using a Arraystar Flash RNA l Labeling kit; Arraystar, Inc.). The labeled cRNA was purified using an RNeasy mini kit (Qiagen, Inc., Valencia, CA, USA). The concentration and specific activity of the labeled cRNAs (pmol Cy3/μg cRNA) were measured using a NanoDrop ND-1000 spectrophotometer. An Agilent Gene Expression Hybridization kit (cat. no. 5188-5242) was purchased from Agilent Technologies, Inc. and each labeled cRNA (1 μg) was fragmented by adding 5 μl 10X blocking agent and 1 μl 25X fragmentation buffer. The mixture was heated at 60˚C for 30 min. Finally, 25 μl 2X GE Hybridization buffer was added to dilute the labeled cRNA. A total of 50 μl hybridization solution was dispensed into the gasket slide and assembled to the lncRNA expression microarray slide. The slides were incubated for 17 h at 65˚C in an Agilent hybridization oven (Agilent Technologies, Inc.). The hybridized arrays were washed, fixed, and scanned using the Agilent DNA microarray scanner (part no. G2505C; Agilent Technologies, Inc.).

Microarray data analysis. Agilent feature extraction software (version 11.0.1.1; Agilent Technologies, Inc.) was used to analyze the acquired array images. Quantile normalization and subsequent data processing were performed using the GeneSpring GX version 12.1 software package (Agilent Technologies, Inc.). Following quantile normalization of the raw data, lncRNAs and mRNAs, for which at least 3 out of 6 samples had flags in Present or Marginal ('All targets value') were selected for further data analysis. Differentially expressed lncRNAs and mRNAs with statistical significance between high-grade ovarian serous cancer and healthy fallopian tube tissue were identified through P-value (<0.05) and false discovery rate (<0.1) filtering. Differentially expressed lncRNAs and mRNAs between the two samples were identified through fold change filtering. Pathway analysis and Gene Ontology (GO) analysis (22,23) were applied to determine

Table I. Primer sequences for lncRNAs and mRNAs.

Gene name	Forward (5'-3')	Reverse (5'-3')
GTSE1-AS1	TCTTAAGCTTCCTGAGGTTGCAC	AGAGGTTTAATTGGCTCACAGTTC
FAS-AS1	CAAAACAGGCTGCTCAAGTTTC	GGGTTGGTGGTACTCGTTCC
AK130076	TGGCCATCCCTACAGTGCTAG	TGGAGTGCAGTGGTGCGATC
RP11-199F11.2	GTGCCTGTAATCCCAGCTATTC	TGAGACGGAGTTTCGTTCTTGTC
AC093818.1	CACTCACCGAAGTCCAGGAAC	GCAGCTTCAGCTCGGACTC
GTSE1	GATGACCCCCAAAACGATG	TTCTGTTGCTCTCCCTTGTTG
FAS	ACACTCACCAGCAACACCAA	TCCTTTCTCTTCACCCAAACA
PTEN	AGACCATAACCCACCACAGC	ACCAGTTCGTCCCTTTCCA
TP53	TAGTGTGGTGGTGCCCTATG	CCAGTGTGATGATGGTGAGG
PDK1	GATGAGTGACCGAGGAGGTG	TAACCAAAACCAGCCAGAGG
GAPDH	CTCAGACACCATGGGGAAGGTGA	ATGATCTTGAGGCTGTTGTCATA

GTSE1-AS1, GTSE1 antisense RNA 1 (head to head); *FAS-AS1*, FAS antisense RNA 1; *GTSE1*, G2 and S-phase expressed 1; *FAS*, Fas cell surface death receptor; *PTEN*, phosphatase and tensin homolog; *TP53*, tumor protein p53; *PDK1*, pyruvate dehydrogenase kinase 1.

which pathways were affected by these differentially expressed lncRNAs and mRNAs. Hierarchical clustering and combined analysis were performed using in-house scripts.

RT-qPCR analysis of lncRNA and mRNA expression. Expression levels of lncRNAs and mRNAs were validated by RT-qPCR analysis among 20 high-grade ovarian serous cancer samples. Total RNA was extracted from the tissue samples using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse-transcribed to cDNA using a TRUEscript 1st strand cDNA synthesis kit (Aidlab Biotechnologies Co., Ltd., Beijing, China) following the manufacturer's protocol. Reactions were incubated for 30 min at 42°C, 5 min at 85°C, and then samples were stored at -20°C. qPCR was performed using 2X SYBR green qPCR mix (Aidlab Biotechnologies Co., Ltd.) in an ABI 7900HT sequence detection machine (Thermo Fisher Scientific, Inc.). The reactions were incubated at 95°C for 3 min, followed by 40 cycles at 95°C for 15 sec and at 60°C for 40 sec. Primer sequences for 5 lncRNAs and 5 mRNAs were designed and synthesized (Table I). GAPDH was used as an internal control. Target and reference (GAPDH) genes were amplified in separate wells and run in triplicate. Statistical analyses of the results were performed using the $2^{-\Delta\Delta Cq}$ relative quantification method (24).

Statistical analysis. SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) were used for statistical analysis. Data are presented as the mean ± standard deviation. The statistical significance of the microarray results was analyzed by fold change and Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

RNA quality control. The integrity of RNAs was assessed by electrophoresis on a denaturing agarose gel. The 28S and 18S ribosomal RNA bands should appear as relatively sharp,
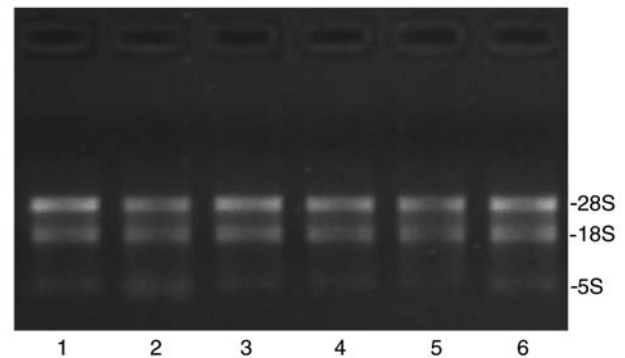

Figure 1. RNA integrity and gDNA contamination test by denaturing agarose gel electrophoresis. The 28S and 18S rRNA bands are clear and intact. Furthermore, the 28S band is twice as intense as the 18S band. Lanes 1-3 represent high-grade ovarian serous cancer tissue samples (A1-A3) while lanes 4-6 represent healthy fallopian tube tissue samples (B1-B3). gDNA, genomic DNA; rRNA, ribosomal RNA.

intense bands. The intensity of the upper band (28S rRNA band) should be ~2x that of the lower band (18S rRNA band). Fig. 1 revealed that the total RNA obtained was of high purity. Total RNA quantification and quality were assured using the NanoDrop ND-1000 spectrophotometer. For spectrophotometry, the optical density A260/A280 ratio should be close to 2.0 for pure RNA (ratios between 1.8 and 2.1 are acceptable). The optical density A260/A230 ratio should be >1.8. In the present study, the total RNA quality of each sample was demonstrated (Table II), suggesting that the samples were appropriate for use in further experiments.

Overview of lncRNA and mRNA profiles. The Arraystar human lncRNA microarray v. 4.0 is designed for the global expression profiling of human lncRNA and protein-coding mRNA transcripts. The array is able to detect a total of 40,173 lncRNAs and 20,730 protein-coding mRNAs. In the present study, ~4,289 differentially expressed lncRNAs and 4,246 differentially expressed mRNAs were detected between high-grade ovarian

Table II. RNA quantification and quality assurance, as assessed using a NanoDrop ND-1000 spectrophotometer.

Sample ID	OD260/280	OD260/230	Concentration (ng/μl)	Volume (μl)	Quantity (ng)	QC result (pass or fail)
A1	2.00	2.24	1,550.05	80	124,004.0	Pass
A2	2.03	2.33	1,175.57	80	94,045.6	Pass
A3	1.99	2.26	888.76	100	88,876.0	Pass
B1	1.97	2.27	594.67	40	23,786.8	Pass
B2	1.89	2.32	420.78	40	16,831.2	Pass
B3	1.89	2.36	357.84	40	14,313.6	Pass

A1-A3: high-grade ovarian serous cancer tissue samples; B1-B3: healthy fallopian tube tissue samples; OD, optical density; QC, quality control.

Table III. The 10 most upregulated and downregulated lncRNAs in high-grade ovarian serous cancer tissues compared with non-tumor tissues, compared by volcano plot.

A, Upregulated lncRNAs

Seq. name	Gene symbol	Fold change	Chromosome	Associated gene name	Associated protein name
TCONS_12_00000435	*XLOC_l2_000324*	81.26	chr1		
NR_002947	*TCAM1P*	62.61	chr17		
NR_002712	*CXCR2P1*	45.67	chr2		
uc002ywy.3	*AK027145*	43.38	chr21	*KCNJ15*	ATP-sensitive inward rectifier potassium channel 15
ENST00000419814	*RP11-131J3.1*	41.20	chr1		
NR_027072	*LINC00189*	38.96	chr21		
ENST00000428667	*AP000695.4*	36.94	chr21	*CLDN14*	Claudin-14
ENST00000470135	*RP5-884M6.1*	35.52	chr7		
ENST00000455309	*AC017002.1*	27.37	chr2		
ENST00000606457	*RP11-1C8.7*	23.40	chr8		

B, Downregulated lncRNAs

Seq. name	Gene symbol	Fold change	Chromosome	Associated gene name	Associated protein name
ENST00000556942	*RP11-356K23.1*	1221.26	chr14	*FOXN3*	Forkhead box protein N3 isoform 1
ENST00000556942	*RP11-356K23.1*	1221.26	chr14	*FOXN3*	Forkhead box protein N3 isoform 2
uc031qjg.1	*AK129935*	845.63	chr12		
ENST00000574178	*RP11-424M24.5*	439.06	chr16		
ENST00000584807	*WI2-1959D15.1*	331.26	chr9	*CR392000.1*	UniProtKB/TrEMBL or E7EUX6
AL049990	*AL049990*	330.93	chr4		
NR_110114	*LOC101927668*	324.98	chr7		
NR_003063	*TUBA4B*	302.06	chr2		
uc002xuq.1	*AK055386*	239.29	chr20		
NR_110916	*LINC01571*	221.41	chr16		

LncRNA, long non-coding RNA.

serous cancer tissue samples and healthy fallopian tube tissue samples, using this fourth-generation lncRNA microarray (fold change, ≥2.0; P<0.05). Among these lncRNAs, 1,511 were upregulated and 2,778 were downregulated. The most upregulated lncRNA was TCONS_l2_00000435 (fold change, 81.26) and the most downregulated lncRNA was RP11-356K23.1 (fold

Table IV. The 10 most upregulated and downregulated mRNAs in high-grade ovarian serous cancer tissues compared with non-tumor tissues, compared by volcano plot.

A, Upregulated mRNAs

Seq. name	Gene symbol	Fold change	Chromosome	Description
NM_153426	*PITX2*	333.42	chr4	Paired-like homeodomain 2
NM_001884	*HAPLN1*	184.94	chr5	Hyaluronan and proteoglycan link protein 1
NM_002125	*HLA-DRB5*	184.27	chr6	Major histocompatibility complex, class II, DR beta 5
NM_021192	*HOXD11*	119.74	chr2	Homeobox D11
NM_014391	*ANKRD1*	112.86	chr10	Ankyrin repeat domain 1 (cardiac muscle)
NM_003108	*SOX11*	111.46	chr2	SRY-box 11
NM_001854	*COL11A1*	106.05	chr1	Collagen, type XI, alpha 1
NM_003469	*SCG2*	93.91	chr2	Secretogranin II
NM_005733	*KIF20A*	91.34	chr5	Kinesin family member 20A
NM_022346	*NCAPG*	74.57	chr4	Non-SMC condensin I complex subunit G

B, Downregulated mRNAs

Seq. name	Gene symbol	Fold change	Chromosome	Description
NM_176813	*AGR3*	2696.97	chr7	Anterior gradient 3, protein disulphide isomerase family member
NM_006408	*AGR2*	1914.59	chr7	Anterior gradient 2, protein disulphide isomerase family member
NM_053285	*TEKT1*	1848.50	chr17	Tektin 1
NM_003357	*SCGB1A1*	1791.03	chr11	Secretoglobin family 1A member 1
NM_031956	*TTC29*	1334.47	chr4	Tetratricopeptide repeat domain 29
NM_001004303	*C1orf168*	1088.35	chr1	Chromosome 1 open reading frame 168
NM_001080537	*SNTN*	1051.19	chr3	Sentan, cilia apical structure protein
NM_000777	*CYP3A5*	1007.52	chr7	Cytochrome P450 family 3 subfamily A member 5
NM_024730	*RERGL*	778.24	chr12	RERG/RAS-like
NM_005634	*SOX3*	752.28	chrX	SRY-box 3

change, 1221.26). In addition, 1,834 mRNAs were upregulated while 2,412 mRNAs were downregulated. The most upregulated mRNA was paired-like homeodomain 2 (fold change, 333.42) and the most downregulated mRNA was anterior gradient 3 (fold change, 2696.97). The 10 most upregulated and down-regulated lncRNAs (Table III) and mRNAs (Table IV) were listed. Distinguishable lncRNA and mRNA expression patterns of the samples are presented via heat maps of the hierarchical clustering (Fig. 2A and B). Reproducible changes in gene expression were observed between the two groups through scatter plots (Fig. 3A and B). LncRNAs and mRNAs with statistically significant differences in expression between the two groups (fold change, ≥2.0; P<0.05) were identified by volcano plot filtering (Fig. 4A and B).

Bioinformatics analysis. Pathway analysis was used to determine biological pathways where differentially expressed genes were significantly enriched, according to the Kyoto Encyclopedia of Genes and Genomes (25), BioCarta (26) and Reactome (27,28) databases (29). Total pathway analysis results revealed that 121 pathways

had significant differences in gene expression between high-grade ovarian serous cancer tissue samples and healthy fallopian tube tissue samples. Of these, 61 pathways were upregulated and 60 were downregulated. The top ten upregulated and downregulated pathways are presented in Fig. 5A and B. The top three upregulated pathways included systemic lupus erythematosus, alcoholism and viral carci-nogenesis. The top three downregulated pathways included drug metabolism-cytochrome P450, cGMP-PKG signaling pathway, and chemical carcinogenesis.

GO analysis was used to analyze the main functions of the differentially expressed genes, according to the GO database. The GO database provides the key functional classifications for the National Center for Biotechnology Information (22), which comprises three structured networks: Biological processes, cellular components and molecular function (30). The most enriched GO terms in each structured network were mitotic cell cycle, chromosome, protein binding, cilium organization, cilium, and calcium ion binding. The first three of these terms were associated with upregulated genes in high-grade ovarian serous cancer tissue samples compared with the healthy
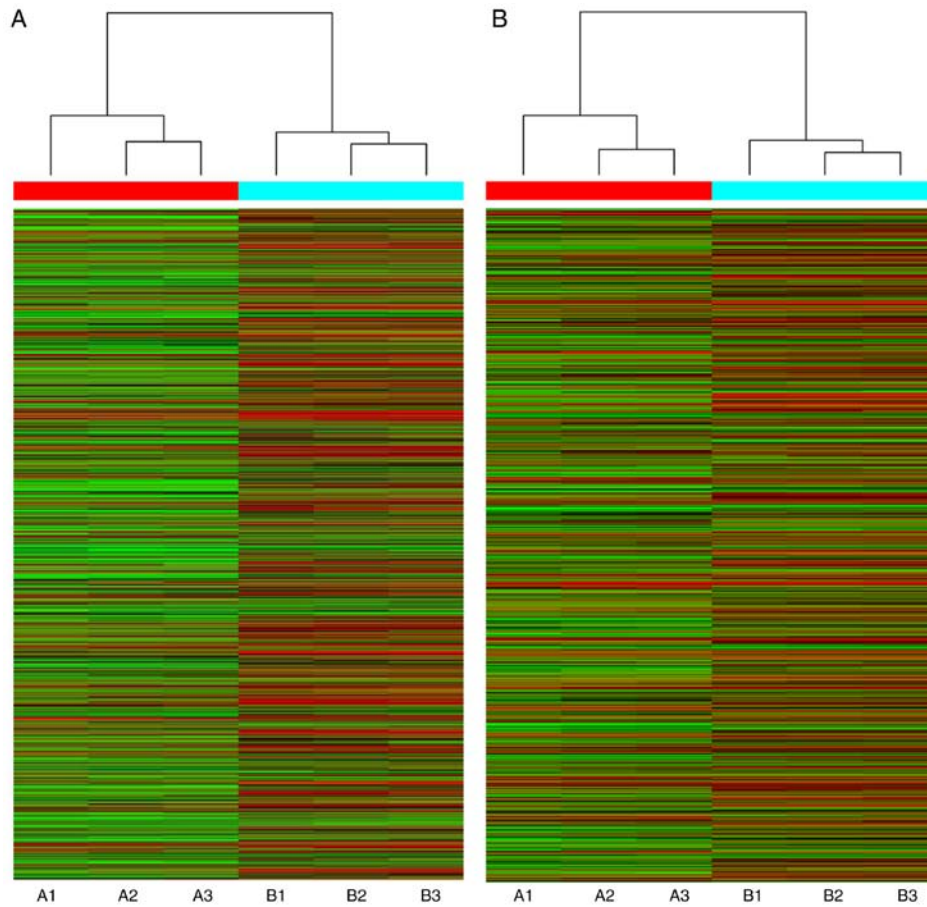
Figure 2. Heat map and hierarchical clustering. Cluster analysis arranges samples into groups based on their expression levels. This analysis was performed to examine distinguishable (A) long non-coding RNA and (B) mRNA expression patterns among the samples. In the dendrogram, red indicates high relative expression and green indicates low relative expression. A1-A3 represent high-grade ovarian serous cancer tissue samples. B1-B3 represent healthy fallopian tube tissue samples.
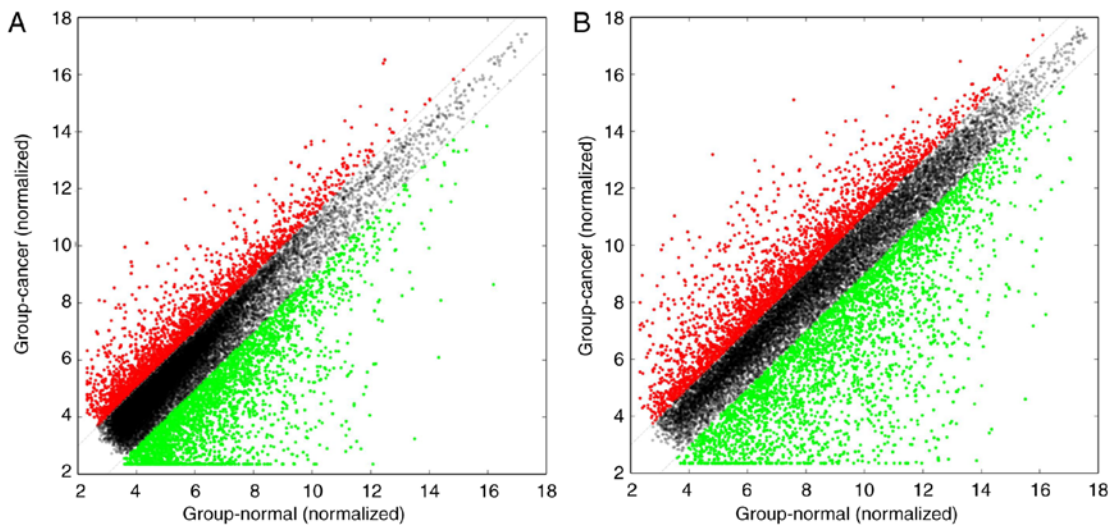

Figure 3. Scatter plots were used to assess variation in (A) long non-coding RNA and (B) mRNA expression between the two groups. The values plotted on the X and Y axes are the averaged normalized signal values in each group (log2 scaled). Red points in the plot indicate >2.0-fold upregulation of expression between the two groups, green points indicate >2.0-fold downregulation of expression, and black points indicate <2.0-fold change in expression.

fallopian tube tissue samples, and the last three terms were associated with downregulated genes. The top ten upregulated and downregulated GO functions of score enrichment terms are presented in Fig. 6.

Subgroup analysis. Subgroup analysis was used to further investigate the associations between lncRNA expression and high-grade ovarian serous cancer. The differentially expressed antisense lncRNAs and their associated mRNAs were
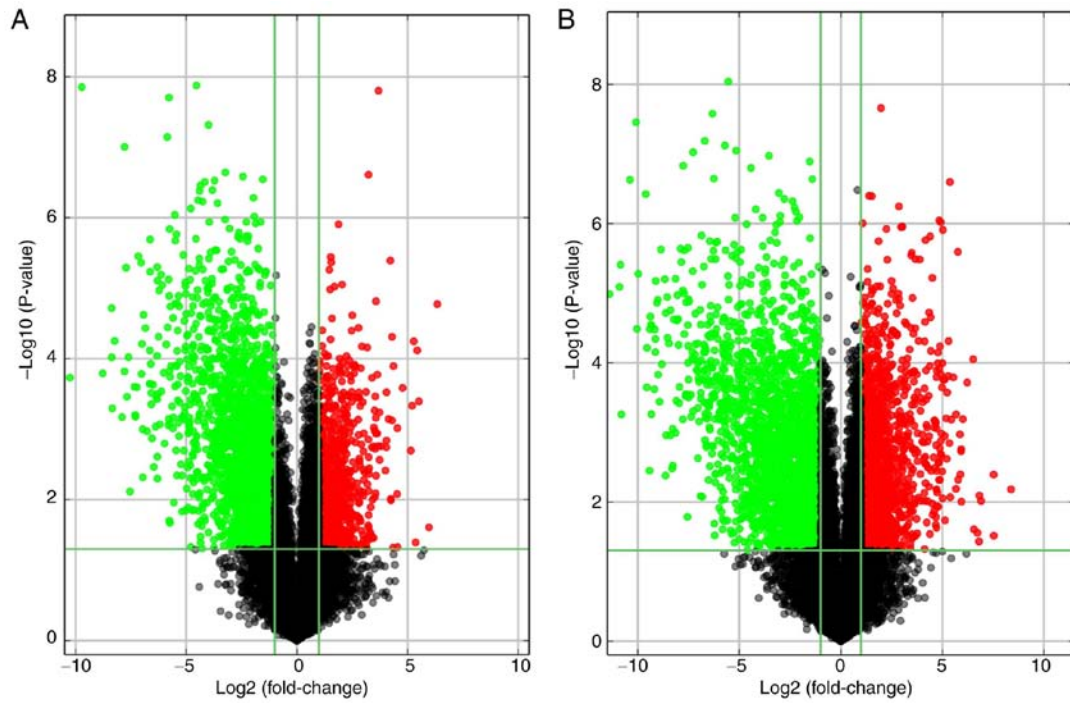
Figure 4. Volcano plot filtering was performed to identify (A) long non-coding RNAs and (B) mRNAs with statistically significant differences in expression. Vertical green lines correspond to 2.0-fold upregulation and 2.0-fold downregulation of expression, and horizontal green lines indicate P=0.05. Thus, the red and green points in the plot indicate >2.0-fold upregulation and downregulation of expression with statistical significance.
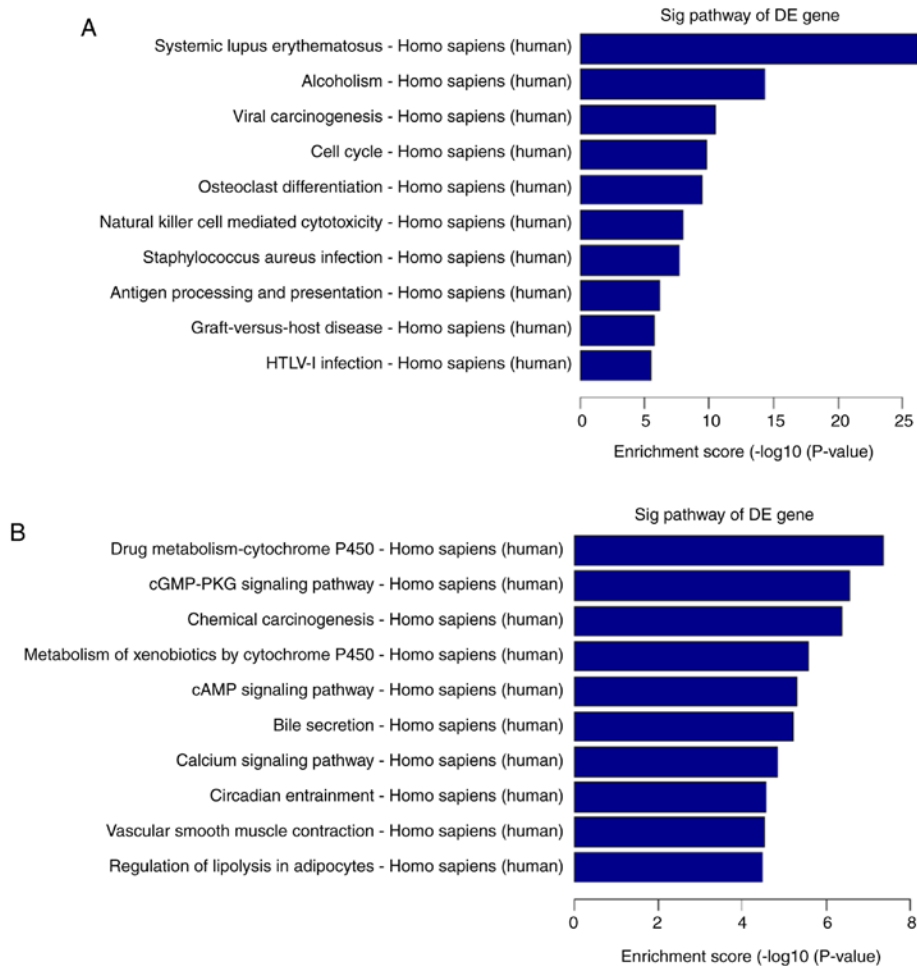
Figure 5. Pathway analysis report. The top 10 significant pathways of (A) upregulated and (B) downregulated genes in high-grade ovarian serous cancer. DE, differentially expressed.
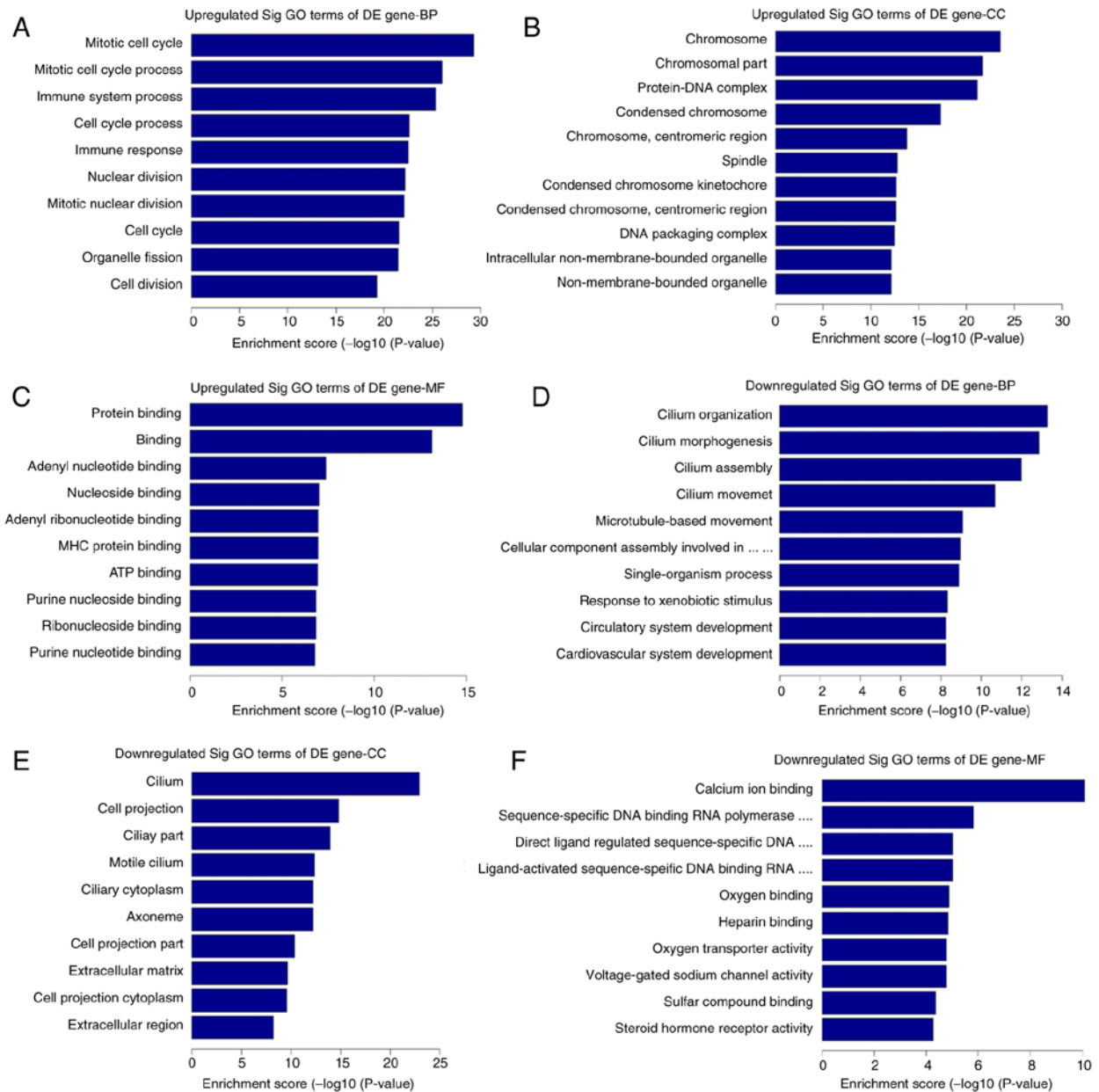
Figure 6. GO analysis report. The GO categories cover three domains: BP, CC and MF. The top 10 upregulated GO functions of the (A) BP, (B) CC and (C) MF domains. The top 10 downregulated GO functions of the (D) BP, (E) CC and (F) MF domains. GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function; DE, differentially expressed.

integrated to infer the function of the lncRNAs. The results revealed that 315 antisense lncRNAs and associated mRNAs were abnormally expressed (fold change, ≥2.0; P<0.05): 51 of the lncRNA-mRNA pairs were regulated in the up-up direction, 200 pairs were regulated in the down-down direction, 30 pairs were regulated in the up-down direction, and 34 pairs were regulated in the down-up direction. A total of 10 differentially expressed antisense lncRNAs and associated mRNAs are presented in Table V. In addition, long intergenic non-coding RNAs (lincRNAs) are of particular interest to the present study as they may be a novel factor associated with cancer progression (31). Statistical analysis of differentially expressed lincRNAs and their nearby mRNAs (distance <300 kb) was conducted. The results revealed that 1,807 lincRNAs and nearby mRNAs were abnormally expressed

(fold change, ≥2.0; P<0.05): 475 of the lincRNA-mRNA pairs were regulated in the up-up direction, 738 pairs were regulated in the down-down direction, 264 pairs were regulated in the up-down direction and 330 pairs were regulated in the down-up direction. A total of 10 differentially expressed lincRNAs and nearby mRNAs are presented in Table VI.

Validation of microarray data with RT-qPCR. To validate the microarray consistency, RT-qPCR was performed. A total of 5 abnormally expressed lncRNAs (*GTSE1-AS1, FAS-AS1, AK130076, RP11-199F11.2* and *AC093818.1*) and their associated mRNAs [G2 and S-phase expressed 1 (*GTSE1),* Fas surface cell death receptor *(FAS),* phosphatase and tensin homolog *(PTEN),* tumor protein p53 *(TP53)* and pyruvate dehydrogenase kinase 1 (*PDK1*)] were selected, and their expression was

Table V. A total of 10 differentially expressed lncRNAs (antisense) and their associated coding gene pairs.

Antisense LncRNAs		Associated mRNAs			Direction (lncRNA-mRNA)
Seq. name	Gene symbol	Nearby gene	Nearby gene symbol	Nearby protein name	
ENST00000373226	RP11-435D7.3	NM_022111	CLSPN	Claspin	Up-up
ENST00000433905	RP4-583P15.10	NM_025224	ZBTB46	Zinc finger and BTB domain containing 46	Up-up
ENST00000435915	RP11-217B7.2	NM_005502	ABCA1	ATP binding cassette subfamily A member 1	Up-up
ciRS-7	ciRS-7	NM_004065	CDR1	Cerebellar degeneration related protein 1	Down-down
ENST00000411847	RP11-301L8.2	NM_021069	SORBS2	Sorbin and SH3 domain containing 2	Down-down
ENST00000412896	AC107218.3	NM_024532	SPAG16	Sperm associated antigen 16	Down-down
ENST00000357045	AC073834.3	NM_152275	TTC30A	Tetratricopeptide repeat domain 30A	Up-down
ENST00000414633	SRGAP3-AS1	NM_014850	SRGAP3	SLIT-ROBO Rho GTPase activating protein 3	Up-down
ENST00000415004	RP11-165F24.3	NM_203447	DOCK8	Dedicator of cytokinesis 8	Down-up
ENST00000424435	RP11-350G8.5	NM_000565	IL6R	Interleukin 6 receptor	Down-up

lncRNA, long non-coding RNA.
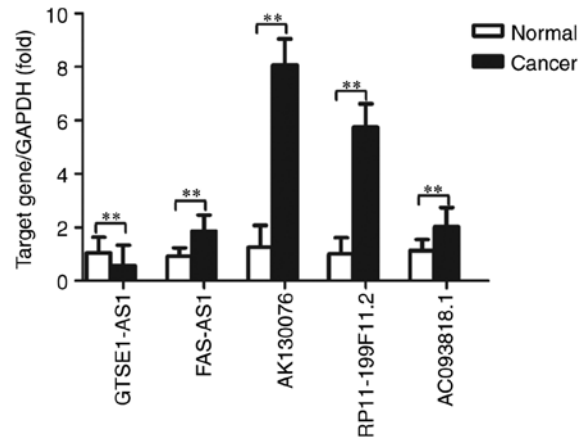
Figure 7. Expression levels of *GTSE1-AS1, FAS-AS1, AK130076, RP11-199F11.2* and *AC093818.1* in high-grade ovarian serous cancer tissue samples and healthy fallopian tube tissue samples, measured by reverse transcription-quantitative polymerase chain reaction. Expression levels were normalized to GAPDH. Data are expressed as the mean ± standard deviation. **P<0.01, with comparisons indicated by lines. *GTSE1-AS1*, GTSE1 antisense RNA 1 (head to head); *FAS-AS1*, FAS antisense RNA 1.
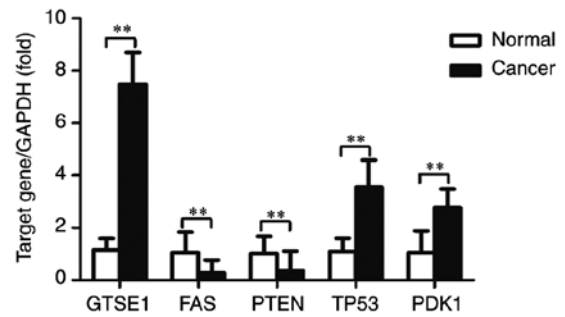
Figure 8. Expression levels of *GTSE1, FAS, PTEN, TP53* and *PDK1* in high-grade ovarian serous cancer tissue samples and healthy fallopian tube tissue samples, measured by reverse transcription-quantitative polymerase chain reaction. Expression levels were normalized to GAPDH. Data are expressed as the mean ± standard deviation. **P<0.01, with comparisons indicated by lines. *GTSE1*, G2 and S-phase expressed 1; *FAS*, Fas cell surface death receptor; *PTEN*, phosphatase and tensin homolog; *TP53*, tumor protein p53; *PDK1*, pyruvate dehydrogenase kinase 1.

analyzed in 20 high-grade ovarian serous cancer tissue samples and healthy fallopian tube tissue samples, using GAPDH as an internal control. These mRNAs have been demonstrated to be associated with cell apoptosis, the p53 signaling pathway, the phosphoinositide 3 kinase (PI3K)/protein kinase B (Akt) signaling pathway, and other mechanisms that are associated with ovarian cancer progression and metastasis (32-35); therefore, our group predicted that these lncRNAs may be involved in the development of ovarian cancer. The results demonstrated that *FAS-AS1, AK130076, RP11-199F11.2* and *AC093818.1* were upregulated and *GTSE1-AS1* was downregulated in high-grade ovarian serous cancer tissues compared with healthy fallopian tube tissues (Fig. 7). For mRNA, *GTSE1, TP53* and *PDK1* were upregulated and *FAS* and *PTEN* were downregulated in high-grade ovarian serous cancer tissues compared with healthy fallopian tube tissues (Fig. 8). The RT-qPCR results matched the microarray data well. Fig. 9 represents the entire workflow. The associations between the expression of

Table VI. A total of 10 cases of differentially expressed long non-coding RNAs (lincRNAs) and nearby coding gene pairs (distance <300 kb).

LincRNAs		Nearby mRNAs			Direction
Seq. name	Gene symbol	Nearby gene	Nearby gene symbol	Nearby protein name	(lncRNA-mRNA)
ENST00000412295	*CTC-338M12.9*	NM_033549	*TRIM41*	Tripartite motif containing 41	Up-up
ENST00000412362	*RP11-236B18.2*	NM_014391	*ANKRD1*	Ankyrin repeat domain 1 (cardiac muscle)	Up-up
ENST00000412427	*RP11-380J14.1*	NM_022089	*ATP13A2*	ATPase type 13A2	Up-up
AF070541	*AF070541*	NM_030632	*ASXL3*	Additional sex combs like 3, transcriptional regulator	Down-down
AF339807	*AF339807*	NM_015567	*SLITRK5*	SLIT and NTRK like family member 5	Down-down
AK023372	*AK023372*	NM_000574	*CD55*	CD55 molecule (Cromer blood group)	Down-down
ENST00000413304	*AC098872.3*	NM_004657	*SDPR*	Serum deprivation response	Dp-down
ENST00000417932	*RP11-10J5.1*	NM_022121	*PERP*	PERP, TP53 apoptosis effector	Up-down
AK055324	*AK055324*	NM_005184	*CALM3*	Calmodulin 3 (phosphorylase kinase, delta)	Down-up
AL049990	*AL049990*	NM_024873	*TNIP3*	TNFAIP3 interacting protein 3	Down-up

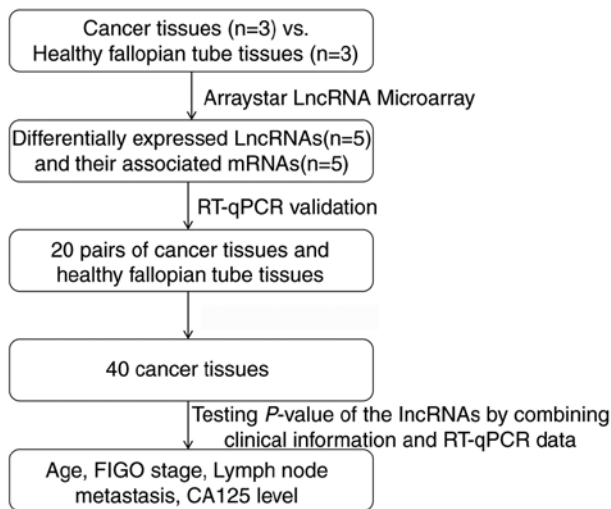LincRNA, long intergenic non-coding RNA.

Figure 9. The entire workflow of the present study. RT-qPCR, reverse transcription-quantitative polymerase chain reaction; FIGO, International Federation of Gynecology and Obstetrics; lncRNA, long non-coding RNA; CA125, cancer antigen 125.

the 5 lncRNAs and clinicopathological parameters in patients with high-grade ovarian serous cancer patients are presented in Table VII. The microarray results of the selected lncRNAs are presented in Table VIII. These lncRNAs were significantly associated with International Federation of Gynecology and Obstetrics (FIGO) stage (36) and lymph node metastases in ovarian cancer. These results suggested that these differentially expressed lncRNAs may be associated with ovarian carcinogenesis and tumor progression.

Discussion

Treatment of high-grade ovarian serous cancer remains a serious challenge and the disease represents a global health problem for women, with a 5-year mortality rate of >70% and a high incidence of metastasis (37). Although the molecular mechanisms underlying high-grade ovarian serous cancer have been investigated, the exact pathogenesis of this disease remains unclear. Therefore, investigation of molecular markers and effective therapeutic strategies targeting high-grade ovarian serous cancer are of great value. At present, an increasing number of studies have indicated that lncRNAs are involved in the regulation of a wide variety of biological processes (38). However, the function that lncRNAs serve in cancer is of particular interest. An increasing number of studies have reported that several types of tumor are associated with differentially expressed lncRNAs, which are involved in occurrence, development, invasion, metastasis, apoptosis and drug resistance, via a series of complicated mechanisms (39,40). For instance, lncRNA *UCA1* was detected in bladder cancer tissues and cell lines, and the expression levels of *UCA1* were significantly higher than that in a control group. Overexpression of *UCA1* increases the proliferation, invasion, and metastasis of bladder cancer cells (41). Hou *et al* (14) discovered that long intergenic non protein-coding RNA, regulator of reprogramming (*LINC-ROR*) was upregulated in breast tumor samples, and ectopic overexpression of *LINC-ROR* increased breast cancer cell migration and invasion. Furthermore, *LINC-ROR* functioned as a competing endogenous RNA to microRNA-205 and induced epithelial-to-mesenchymal transition (14). In addition, dysregulation of lncRNAs, including

Table VII. Associations between long non-coding RNA expression and clinicopathological parameters in 40 patients with high-grade ovarian serous cancer.

Parameter	Age (years)		FIGO stage		Lymph node metastasis		CA125 level (U/ml)	
	<50	≥50	I-II	III-IV	Negative	Positive	<600	≥600
GTSE1-AS1								
High no. cases	6	8	5	4	6	4	10	6
Low no. cases	10	16	16	15	17	13	15	9
P-value	0.548		<0.01		<0.01		0.432	
FAS-AS1								
High no. cases	9	15	17	14	16	12	17	8
Low no. cases	7	9	4	5	7	5	8	7
P-value	0.433		<0.01		<0.01		0.694	
AK130076								
High no. cases	10	16	18	15	18	12	17	10
Low no. cases	6	8	3	4	5	5	8	5
P-value	0.249		<0.01		<0.01		0.167	
RP11-199F11.2								
High no. cases	10	15	16	16	20	11	14	9
Low no. cases	6	9	5	3	3	6	11	6
P-value	0.726		<0.01		<0.01		0.259	
AC093818.1								
High no. cases	11	14	15	14	17	14	16	8
Low no. cases	5	10	6	5	6	3	9	7
P-value	0.673		<0.01		<0.01		0.464	

FIGO, International Federation of Gynecology and Obstetrics; CA125, cancer antigen 125; *GTSE1-AS1*, GTSE1 antisense RNA 1 (head to head); *FAS-AS1*, FAS antisense RNA 1; High no. cases, numbers of specimens with high expression of associated genes in high-grade ovarian serous cancer (compared with the expression level of associated genes in healthy fallopian tube tissue samples); Low no. cases, numbers of specimens with low expression of associated genes in high-grade ovarian serous cancer (compared with the expression level of associated genes in healthy fallopian tube tissue samples).

Table VIII. Microarray results of the selected five long non-coding RNAs.

A, Downregulated

Seq. name	Gene symbol	Fold change	Chromosome	Associated gene name	Associated protein name
NR_024009	*GTSE1-AS1*	3.11	chr22	*GTSE1*	G2 and S phase-expressed protein 1

B, Upregulated

Seq. name	Gene symbol	Fold change	Chromosome	Associated gene name	Associated protein name
NR_028371	*FAS-AS1*	2.01	chr10	*FAS*	Tumor necrosis factor receptor superfamily member 6 isoform 1 precursor
uc001kfc.1	*AK130076*	6.08	chr10	*PTEN*	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein Phosphatase PTEN
ENST00000571370	*RP11-199F11.2*	5.87	chr17	*TP53*	Cellular tumor antigen p53 isoform a
ENST00000436922	*AC093818.1*	2.54	chr2	*PDK1*	Pyruvate dehydrogenase kinase, isozyme 1

HOTAIR, metastasis associated lung adenocarcinoma transcript 1, antisense non-coding RNA in the INK4 locus, growth arrest-specific 5, cervical carcinoma expressed PCNA regulatory lncRNA, H19, imprinted maternally expressed transcript (non-protein coding), and lncRNA-activated by TGFβ, has been demonstrated to exacerbate several human cancers, including small-cell lung cancer (12), prostate cancer (42), gastric cancer (15), colorectal cancer (43), cervical cancer (44), liver cancer (45) and pancreatic cancer (46). Therefore, lncRNAs represent potential indicators of tumor prognosis and diagnosis.

Usually, searching for differentially expressed lncRNAs with high-throughput microarray technologies in cell lines and tissues is the first step in the study of genes. Zhou *et al* (47) investigated differences in lncRNA and mRNA expression profiles between hypopharyngeal squamous cell carcinoma tissues and adjacent non-tumor tissues by microarray analysis, and identified a series of significantly dysregulated lncRNAs and mRNAs. These results laid the foundation for their following research. Similar studies have been carried out in pancreatic cancer, endometrial cancer and nasopharyngeal carcinoma (48-50).

In the present study, three pairs of high-grade ovarian serous cancer tissue samples and healthy fallopian tube tissue samples were used to investigate the differences in lncRNA and mRNA expression profiles through microarray analysis. The results revealed that the expression profiles of lncRNAs and mRNAs in high-grade ovarian serous cancer tissues were significantly altered. In addition, compared with the number of upregulated genes, the number of downregulated genes was larger, indicating that this may influence the occurrence and development of high-grade ovarian serous cancer. These differentially expressed genes were subsequently organized into hierarchical categories based on heat maps of hierarchical clustering, and the differences in lncRNA and mRNA expression between the two groups were analyzed by scatter plot and volcano plot filtering. Furthermore, GO and pathway analysis were performed to obtain information on the biological functions and potential mechanisms underlying the action of these differentially expressed lncRNAs. However, more detailed research should be performed to investigate the specific mechanisms existing between these pathways, networks and genes.

Antisense lncRNA has been the subject of intense research among lncRNAs (51). In total, >30% of annotated human transcripts have corresponding antisense lncRNAs. These antisense lncRNAs regulate the corresponding sense lncRNAs at transcription or post transcription level through a variety of mechanisms, and serve an important biological function. In addition, lincRNAs are a subject of particular interest to the present study (52). Multiple studies have demonstrated that a common and important function of lncRNAs is to alter the expression of nearby coding genes by affecting their transcription (53-55). Therefore, in the present study, differentially expressed antisense lncRNAs, lincRNAs, and their associated mRNAs were integrated in order to infer the function of lncRNAs in lncRNA-mRNA coexpression, which may predict the target genes of lncRNAs. Subgroup analysis results identified 315 abnormally expressed antisense lncRNAs and associated mRNAs, as well as 1807 abnormally expressed lincRNAs and nearby mRNAs. However, the function of this lncRNA-mRNA coexpression requires further research.

To confirm the microarray consistency, 5 differentially expressed lncRNAs (*GTSE1-AS1, FAS-AS1, AK130076, RP11-199F11.2* and *AC093818.1*) and their associated mRNAs (*GTSE1, FAS, PTEN, TP53* and *PDK1*) were selected to verify expression consistency by RT-qPCR. *FAS* has been reported to be associated with ovarian cancer cell apoptosis (32). *PTEN* serves an important biological function in ovarian cancer cell growth, proliferation, and migration (34). *TP53* is a common tumor suppressor gene, which influences ovarian cancer cell proliferation and cell cycle through the p53 signaling pathway (33). PDK1 is involved in the development and drug resistance mechanisms of ovarian cancer by regulating the PI3K/Akt signaling pathway (35). The present study revealed that *GTSE1, FAS, PTEN, TP53*, and *PDK1* are the associated mRNAs of *GTSE1-AS1, FAS-AS1, AK130076, RP11-199F11.2* and *AC093818.1*, suggesting that these lncRNAs may serve a role in the development of ovarian cancer. In particular, the expression of 4 lncRNAs (*FAS-AS1, AK130076, RP11-199F11.2* and *AC093818.1*) was significantly increased in high-grade ovarian serous cancer tissues compared with healthy fallopian tube tissues, while the expression of *GTSE1-AS1* was significantly lower. The expression of 3 associated mRNAs (*GTSE1, TP53* and *PDK1*) was significantly increased in high-grade ovarian serous cancer tissues; that of *FAS* and *PTEN* was significantly decreased compared with normal tissues. These results are consistent with the microarray data and reflect the variable expression of lncRNAs and mRNAs in different tissues. Furthermore, the expression of the selected lncRNAs was significantly associated with ovarian cancer FIGO stages and lymph node metastases in 40 patients with high-grade ovarian serous cancer. These results provide more evidence that these differentially expressed lncRNAs may be associated with ovarian carcinogenesis and tumor progression. Although the biological functions of a large number of genes remain unclear, the data from the present study may be useful for further studies on the pathogenesis and underlying molecular mechanisms of high-grade ovarian serous cancer. Multiple further studies are necessary to strengthen the association between lncRNAs and high-grade ovarian serous cancer. In consequent work, our group will further explore gene expression and clinical data in high-grade ovarian serous cancer according to The Cancer Genome Atlas and Gene Expression Omnibus databases, and analyze the associations with the prognosis of patients with high-grade ovarian serous cancer. Further experiments, including immunohistochemistry, western blot analysis and other *in vivo* and *in vitro* experiments will be performed to identify the specific molecular mechanisms and biochemical functions between lncRNAs and high-grade ovarian serous cancer.

In conclusion, to the best of our knowledge, the present study was the first to examine differences in lncRNA and mRNA expression profiles between high-grade ovarian serous cancer tissues and healthy fallopian tube tissues using microarray analysis. According to the present study, 4,289 lncRNAs and 4,246 mRNAs were differentially expressed in high-grade ovarian serous cancer tissues, and may serve a key function in the occurrence and development of high-grade ovarian serous cancer. Furthermore, bioinformatics analyses were applied to determine the potential functions of these abnormally

expressed genes. In the near future, our group will conduct more detailed research on several lncRNAs, and identify their specific molecular mechanisms and biochemical functions for the purpose of providing effective methods for the diagnosis and therapy of high-grade ovarian serous cancer.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL and HJ conceived and designed the study. ZC and YH collected the specimens. YL, HJ, XW and LW performed the experiments. YL and HJ wrote the paper. ZC, YH, XW and LW reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects provided signed statements of written informed consent. All experimental procedures were approved by the Institutional Review Board of the Ethics Board of the Affiliated Hospital of Qingdao University (Qingdao, China).

Consent for publication

All patients provided written informed consent for the publication of their data.

Competing interests

The authors declare that they have no competing interests.

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