

Identification of key genes and pathways between type I and type II endometrial cancer using bioinformatics analysis

KAI ZHANG*, HUIYANG LI*, YE YAN, YUQIN ZANG, KE LI, YINGMEI WANG and FENGXIA XUE

Department of Gynecology and Obstetrics, Tianjin Medical University General Hospital, Tianjin 300052, P.R. China

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Abstract. Endometrial carcinoma (EC) is a common malignant neoplasm of the female reproductive tract. The malignant degree of type II EC is much greater than that of type I EC, usually presenting with a high recurrence rate and a poor prognosis. Therefore, the present study aimed to examine the principal genes associated with the degree of differentiation in type I and type II EC and reveal their potential mechanisms. Differentially expressed genes (DEGs) were selected from the gene expression profiles derived from The Cancer Genome Atlas. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted. In the present study, the KEGG pathway enrichment analysis revealed that 5,962 upregulated DEGs were significantly enriched in the 'p53 signaling pathway' and involved in 'lysine degradation'. In addition, 3,709 down-regulated DEGs were enriched in 'pathways in cancer', as well as 'tight junction regulation', the 'cell cycle' and the 'Wnt signaling pathway'. The 13 top hub genes MAPK1, PHLPP1, ESR1, MDM2, CDKN2A, CDKN1A, AURKA, BCL2L1, POLQ, PIK3R3, RHOQ, EIF4E and LATS2 were identified via the protein-protein interaction network. Furthermore, the OncoPrint algorithm from cBioPortal declared that 25% of EC cases carried genetic alterations. The altered DEGs (MAPK1, MDM2, AURKA, EIF4E and LATS2) may be involved in tumor differentiation and may be valuable diagnostic biomarkers. In conclusion, a number of principal genes were identified in the present study that may be determinants of poorly differentiated type II EC carcinogenesis, which may contribute to future research into potential molecular mechanisms. In addition,

these genes may help identify candidate biomarkers and novel therapeutic targets for type II EC.

Introduction

Endometrial carcinoma (EC) occurs in the endometrium and is one of the most common female gynecological tumors. It was estimated that >63,230 new cases of EC and nearly 11,350 deaths from EC occurred in 2018 in the USA (1). EC usually includes two types based on its estrogen dependency (2). Generally, the majority of ECs that occur in postmenopausal women are type I, with an increased dependency on estrogen. The prognosis of type I EC is good, and this type accounts for 70-80% of ECs (3). By contrast, type II EC usually has a poor prognosis. Surgery is the primary treatment for EC that occurs in postmenopausal women. Despite successful surgical intervention, the recurrence rate within 5 years for type II EC is 10-15%, with poor results and short survival time (4).

Similar to that of other tumors, the process of EC initiation, development and metastasis involves genetic aberrations, and changes in the tumor microenvironment (5). The literature has also reported that the development and occurrence of EC are closely associated with a variety of genes and cellular pathways (6,7). However, there is currently little literature available regarding the treatment of type II EC due to the complex nature of the molecular mechanisms and the unfavorable biological behavior. Thus, it is important to study and understand the cellular mechanisms by which type II EC proliferates or initiates apoptosis when searching for diagnostic tools and measures. The present study aimed to identify the difference between type I and type II EC at the genetic level, and to identify candidate biomarkers or therapeutic targets for type II EC.

In life sciences, high-throughput sequencing analysis of gene expression has become increasingly valued as a promising tool for the early diagnosis and grading of cancer, the prediction of prognosis and the discovery of new anticancer targeted drugs. In the present study, by applying genomic techniques, particularly large-scale genome sequencing analyses, the genomic variation map of human EC was drawn and systematically analyzed to find all the minor mutations of carcinogenic and tumor suppressor genes, which can improve the ability to diagnose, treat and prevent cancer. In the present analysis, 177 samples were selected that contained 20,531 gene expression profiles from The Cancer Genome Atlas (TCGA) database.

Correspondence to: Professor Yingmei Wang or Professor Fengxia Xue, Department of Gynecology and Obstetrics, Tianjin Medical University General Hospital, 154 Anshan Road, Heping, Tianjin 300052, P.R. China
E-mail: wangyingmei1978@126.com
E-mail: fengxiaxue1962@gmail.com

*Contributed equally

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Morpheus (<https://software.broadinstitute.org/morpheus/>) was utilized to select differentially expressed genes (DEGs). Gene Ontology (GO) (8) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (9) pathway enrichment analyses were then performed to investigate the biological processes, functions and pathways associated with the selected DEGs. Next, the protein-protein interaction (PPI) network was investigated via Cytoscape v3.6.1 (<http://www.cytoscape.org/>) to identify key genes involved in type I and type II EC. In addition, the overall survival (OS) of these the dominant genes was analyzed using the Kaplan-Meier method with GraphPad Prism software version 6.0 (GraphPad Software, Inc.). Finally, the potential associations between genes were analyzed using a visual image according to TCGA database. New clues were identified to guide treatment, with gene-targeted therapy also expected to be an effective treatment for type II EC.

Materials and methods

Identification of DEGs from TCGA. The gene expression profiles of EC were collected from the TCGA (10) database through cBioPortal (<https://www.cbioportal.org/>). The Uterine Corpus Endometrial Carcinoma (TCGA, Provisional) which is updated regularly, was downloaded (11,12); it contained the genetic information and clinical data of 547 patients. In total, 177 EC specimens, which included 119 subtype I (endometrioid endometrial adenocarcinoma) and 58 subtype II (serous endometrial adenocarcinoma) samples and corresponded to complete clinical basic information, were included. Thus, the group of 177 cases was a subset of the 547 cohort. The clinical data of the patients, including the demographic features, International Federation of Gynecology and Obstetrics (FIGO) stage (13), and histological type according to the World Health Organization 2014 classification (14), as well as performance status, were retrospectively analyzed. OS and progression-free survival (PFS) were assessed using Kaplan-Meier analyses for the 119 patients with type I disease and the 58 patients with type II disease. Next, the gene expression profile data of type I and type II patients were downloaded from TCGA database and saved in a file, which was uploaded to the Morpheus online tool, which is a versatile matrix visualization and analysis software. A series of processing selection operations were performed, including 'Annotate Selection', 'Maker Selection' and 'T-test'. Finally, the results were presented in the form of a heat map and downloaded. Differential genes with P-values <0.05 were screened by heat maps using Morpheus online tools.

Analysis of the DEGs with GO and KEGG online tools. The Database for Annotation, Visualization and Integrated Discovery (DAVID; <https://david.ncifcrf.gov/>) is a bioinformatics database that integrates biological data and analysis tools to deliver systematic, comprehensive bio-functional annotation information for large-scale gene or protein lists. KEGG (<https://www.genome.jp/>) is a database that integrates information on genomic, chemical and systemic functions to reveal the genetic and chemical blueprints of life processes (15). In addition, KEGG has powerful capabilities that use graphics to introduce numerous metabolic pathways and associations between pathways. The DAVID online software analyzed the potential pathways of the core genes with P-values <0.05.

PPI network construction and module analysis. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; <http://string-db.org/>) is a tool that studies genetic interactions and can assess PPI (16). Therefore, the STRING database (version 10.5) in Cytoscape (version 3.6.1) (17) was used to identify and map potential associations between the DEGs. The cut-off criteria were a confidence score ≥ 0.4 and a maximum number of iterations equal to 0. Modules in the PPI network were extracted from Cytoscape using the MCODE, with the following criteria: Degree cut-off, 2; node score cut-off, 0.2; k-core, 2; and maximum depth, 100. On this basis, only modules with a score >5 were selected for further function and pathway enrichment analyses. The DAVID tool aided the analysis of the pathways between genes within these modules. In addition, the potential associations between the 13 core genes were assessed using KEGG and GO analysis. These genes with confidence scores between >0.4 and <5 maximum number of interactions were assigned to STRING.

Potential molecular mechanisms of the top 13 aberrantly expressed genes in EC. cBioPortal (<https://www.cbioportal.org/>) is a database with integrated genetic data, including DNA mutations, methylations, gene amplifications, homozygous deletions, protein alterations and phosphorous abundance. The present study contains results from 10 published tumor studies and >20 results from TCGA. Similarly, the Uterine Corpus Endometrial Carcinoma (TCGA, Provisional) was selected for further analysis in the cBioPortal database. Therefore, possible genetic alterations of the top 13 aberrantly expressed genes in EC were summarized using the visual cBioPortal v1.11.3 analysis tool, and the clinical value following genetic alterations were further analyzed.

Statistical analysis. All statistical analyses were performed using SPSS software (version 22.0; IBM Corp.). Comparisons of the demographics and general characteristics of the EC sample groups were performed using the χ^2 and Fisher's exact tests. A paired Student's t-test was used to analyse quantitative data. The survival rate was estimated using the Kaplan-Meier method and the comparison between the type I and type II groups was calculated using the log-rank test. Adjusted P-value was used to find genuine tendencies towards co-occurring genes with very low error rates using the Benjamini-Hochberg false discovery rate (FDR) correction procedure. P<0.05 was considered to indicate a statistically significant result.

Results

Identification of the DEGs. The heat maps from the Morpheus online tool were used to compare the data of the type I samples with that of the type II samples, and DEGs were determined using the threshold of P<0.05. In total, 9,671 genes were screened as DEGs from the heat maps. Among them, 5,962 genes were identified as being upregulated, whereas 3,709 were downregulated in type I samples when compared with type II samples. The expression heat maps revealing the top 200 genes in the matched 9,671 DEGs are presented in Fig. 1, and detailed information is also presented in Table SI.

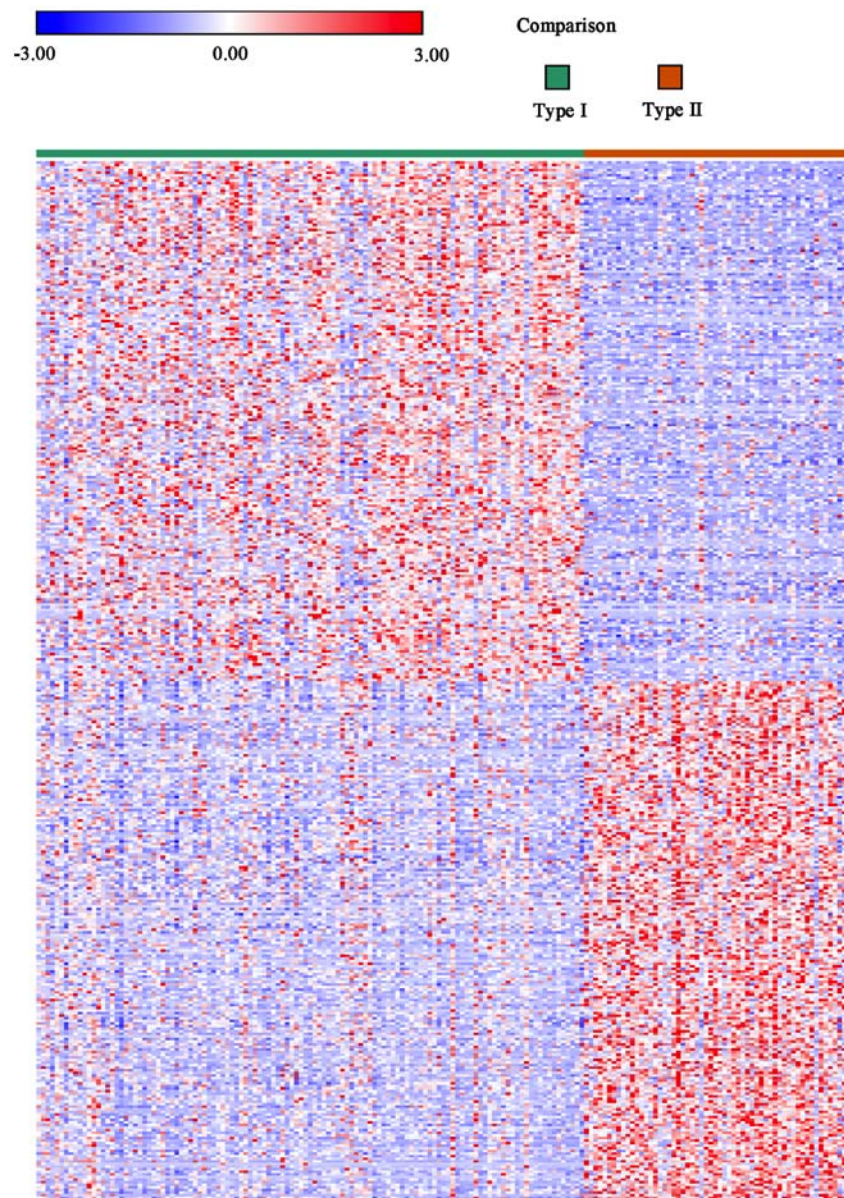


Figure 1. Heat map of the top 200 differentially expressed genes. Red indicates upregulated genes and blue indicates downregulated genes. The top 200 upregulated DEGs and the top 200 downregulated DEGs in type I EC compared with type II EC are shown. DEGs, differentially expressed genes; EC, endometrial carcinoma.

Demographics and clinical characteristics of patients with type I and type II EC. The patients were subdivided into type I and type II groups according to their histopathological type following surgery. In the present study, 119 cases of pathologically confirmed endometrioid adenocarcinoma were selected as the type I group and 58 cases of serous carcinomas were selected as the type II group. There were significant differences in menopausal stage, histological subtype, FIGO stage and vital status. However, there were no significant differences in age, body mass index or ethnicity between the aforementioned two groups. The demographics and clinical characteristics of these two groups are summarized in Table I. The Kaplan-Meier analysis curves of OS of the two groups are presented in Fig. 2A. A significant statistical difference was observed in the OS between the two groups ($P=0.022$), with the type I group experiencing increased survival times, whereas no significant difference was revealed in the

PFS time between the two groups, as presented in Fig. 2B ($P=0.192$).

GO function and KEGG pathway enrichment analyses. All DEGs were uploaded to the functional annotation tool DAVID Bioinformatics Resources (version 6.7), to identify the over-represented GO categories and KEGG pathways. From the GO analysis, the top 5 biological process (BP), cell component and molecular function results are presented in Table II according to their P-values in reverse order.

The results of the GO analysis revealed that the upregulated DEGs were primarily enriched in the BP for 'positive regulation of apoptosis', 'positive regulation of programmed cell death' and 'positive regulation of cell death', as well as 'induction of apoptosis by intracellular signals' and 'fatty acid metabolic process'. The downregulated DEGs focused on 'transcription, DNA-dependent pathways', 'RNA biosynthetic

Table I. Comparison of the demographics and general characteristics of endometrial carcinoma sample groups.

Characteristics	Type I	Type II	P-value
Total patients, n	119	58	
Age range, years	33-88	51-90	0.068
BMI	35.29±19.48	30.02±7.90	0.065
Menopausal, n (%)			0.019
Pre-	14 (11.76)	0 (0.00)	
Post-	96 (80.67)	51 (87.93)	
Data not available	9 (7.56)	7 (12.07)	
Ethnicity, n (%)			0.466
Caucasian	58 (48.74)	34 (58.62)	
African-American	43 (36.13)	17 (29.31)	
Other ^a	18 (15.13)	7 (12.07)	
Histological subtype, n (%)			0.001
Grade 1	13 (10.92)	1 (1.72)	
Grade 2	19 (15.97)	1 (1.72)	
Grade 3	87 (73.11)	56 (96.55)	
FIGO stage, n (%)			<0.001
I	75 (63.03)	24 (41.38)	
II	19 (15.97)	4 (6.90)	
III	22 (18.49)	23 (39.66)	
IV	3 (2.52)	7 (12.07)	
Vital status, n (%)			0.022
Alive	102 (85.71)	41 (70.69)	
Dead	17 (14.29)	17 (29.31)	

^aIncluding Asian, Native Hawaiian or Pacific Islander and American-Indian or Native Alaskan. FIGO, International Federation of Gynecology and Obstetrics; BMI, body mass index.

process', 'apoptosis', 'programmed cell death' and 'apoptotic mitochondrial changes'. It was revealed that the upregulated DEGs were significantly enriched in the 'p53 signaling pathway' and 'lysine degradation' processes, while the downregulated DEGs were enriched in 'pathways in cancer', 'tight junction' regulation, the 'cell cycle', the 'Wnt signaling pathway', 'chronic myeloid leukemia' development and 'small-cell lung cancer' development according to the results of the KEGG pathway enrichment analysis (Table III).

Screening for core genes and modules via the PPI network. An MCODE plug-in was used in Cytoscape to build a PPI network with 212 nodes and 380 edges (Fig. 3A). Using the Network Analyzer tool, the top 13 hub DEGs with the highest node degrees were obtained (Table IV). As presented in Table IV, the hub genes were MAPK1, PHLPP1, ESR1, MDM2, CDKN2A, CDKN1A, AURKA, BCL2L1, POLQ, PIK3R3, RHOQ, EIF4E and LATS2. A PPI network of the top 13 central genes with the highest connectivity was established through the STRING protein analysis (Fig. 3B).

In addition, the MCODE plug-in extracted an important module with a score >4, and then performed function and pathway enrichment analyses (Fig. 4A). This module

consisted of 10 nodes and 41 edges, which were primarily involved in the 'positive regulation of apoptosis', 'positive regulation of programmed cell death' and 'positive regulation of cell death' (Table V). Three other modules were also revealed (Fig. 4B-D), whose further function and pathway enrichment analysis data were not available in the GO and KEGG databases.

Top 13 expression genes in EC. The OncoPrint module in cBioPortal, an online tool, was used to analyze genetic alterations, and showed that 25% (136/547) of the cases had undergone genetic changes (Fig. 5A). The types of genetic alterations included amplification, deep deletion and mRNA upregulation. Notably, patients with genetic alterations had shorter OS and PFS times than those without alterations (log rank, $P=0.0179$; Fig. 5B; and log rank, $P=0.0379$; Fig. 5C, respectively). Furthermore, as presented in Table VI, there were 6 pairs of genes identified via cBioPortal with significant tendencies towards co-occurrence. These genes were PHLPP1 and LATS2, POLQ and LATS2, PHLPP1 and POLQ, ESR1 and CDKN1A, PHLPP1 and PIK3R3, and POLQ and ESR1.

Discussion

EC is a common malignant neoplasm of the female reproductive tract (1). The histological type of disease, determined postoperatively, has a crucial impact on the survival and prognosis of patients with EC. Pathologically determined serous carcinoma usually leads to a poor prognosis with metastasis and more difficult surgery. Furthermore, the 5-year survival rate only reaches 25-30% (18). Consequently, a comprehensive understanding of the molecular mechanism behind the occurrence and development of EC is of importance for management and therapy, as is the development of novel targeted therapies to improve OS time. With the widespread use of next-generation sequencing technologies and microarrays, the expression levels of genes in the human genome are openly included in public databases. Therefore, the molecular mechanisms of different pathological types of EC and microarray data can be investigated in order to facilitate the genetic research of EC.

In the present study, it was observed through GO analysis that upregulated DEGs were primarily enriched in the 'positive regulation of apoptosis', 'positive regulation of programmed cell death', 'positive regulation of cell death', 'induction of apoptosis by intracellular signals' and 'fatty acid metabolic process', and that the downregulated DEGs were primarily involved in 'transcription, DNA-dependent pathways', 'RNA biosynthetic process', 'apoptosis', 'programmed cell death' and 'apoptotic mitochondrial changes'. These results are compatible with the suggestion that the main causes of tumor development and metastasis are defective functions in the regulation of apoptosis and cell death regulators (19). The metabolic patterns, such as that of glycolysis, oxidative phosphorylation, amino acid metabolism, fatty acid metabolism and nucleic acid metabolism, are significantly altered during cell carcinogenesis. Among these processes, fatty acid metabolism is essential for energy storage, membrane proliferation and the synthesis of signaling molecules (20). Thus, studying the mechanism of *de novo* fatty acid synthesis and its association with the development and progression of tumors

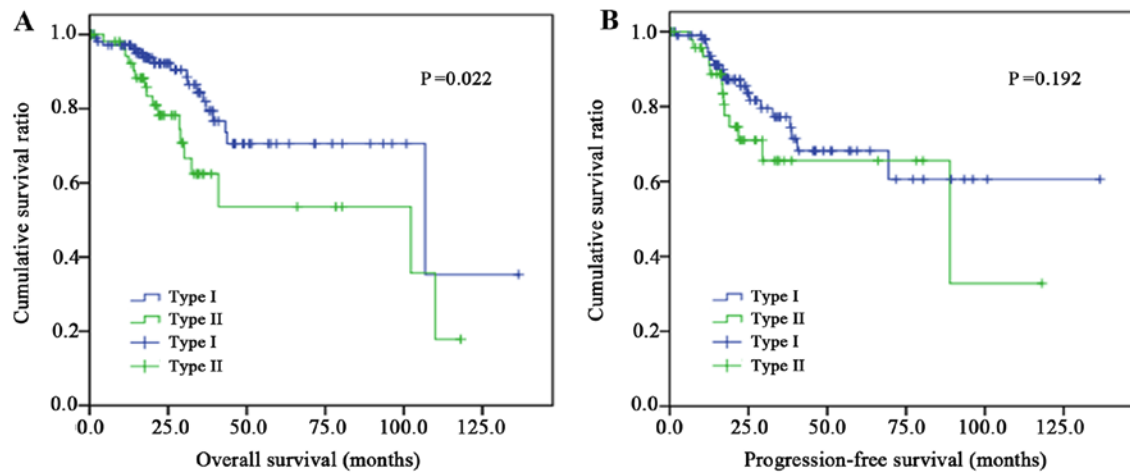


Figure 2. Comparison of overall survival and progression-free survival in patients with type I and type II endometrial cancer. (A) Kaplan-Meier analysis curves of overall survival of 119 patients in the type I group versus 58 patients in the type II group. Significant statistical difference was noted in the overall survival times between the two groups ($P=0.022$). (B) Kaplan-Meier analysis curves of progression-free survival of 119 patients in the type I group versus 58 in the type II group. There was also no statistical difference identified in the progression-free survival times between these two groups ($P=0.192$).

may be a new idea for improving tumor diagnosis, prevention and treatment. A previous study revealed that the risk of EC is significantly reduced by the consumption of ω -3 polyunsaturated fatty acids (PUFAs) in high-fat diets. However, the underlying molecular mechanism of action of PUFAs in EC is not well understood (21). Furthermore, the latest research has also demonstrated that ω -6 is strongly associated with the risk of breast cancer (22,23).

In addition, upregulated DEGs are primarily involved in the 'p53 signaling pathway' and the 'lysine degradation' processes in the KEGG pathway enrichment analysis. Previous studies have revealed that markers of the p53 pathway improved the stratification of EC and can provide novel insights into the effect of this pathway in the morphological classification of high-risk EC (24,25). Furthermore, the phenomenon that the p53 signaling pathway is disturbed in EC has been widely noted. In addition, lysine-specific demethylase 1 (LSD1) plays a vital role in the regulation of chromatin and can affect the occurrence and development of many types of malignant tumor by regulating the proliferation, invasion and metastasis of tumor cells. Therefore, the special role of LSD1 allows for it to become a new antitumor target (26). The downregulated DEGs were enriched in 'pathways in cancer', 'tight junction' regulation, the 'cell cycle', the 'Wnt signaling pathway', 'chronic myeloid leukemia' development' and 'small-cell lung cancer' development. As reported in the literature, alteration of pathways such as the Janus kinase/signal transducer and activator of transcription proteins signaling pathway, the Wnt signaling pathway and the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin pathway were verified in a number of different types of cancer (27,28). For example, stabilizing DVL3 expression can activate the Wnt/ β -catenin signaling pathway in hepatocellular carcinoma (29), and E2F3 expression, as a potent transcriptional inducer of cell-cycle progression, can promote non-small cell lung cancer progression through the microRNA-377-3p-E2F3 pathway (30). These altered DEGs may be co-expressed in different types of cancer and participate in tumorigenesis, such as that of chronic myeloid leukemia and small cell

lung cancer, according to KEGG pathway analysis. In the multi-stage development of cancer, the imbalance of the equilibrium steady state of the activity of signaling pathways responsible for cell cycle regulation and division will lead to the inhibition of apoptosis and the enhancement of cell proliferation (31). Tight junctions are protein structures that control the transport of water, ions and macromolecules across cell layers (32). Previous studies have demonstrated that low levels of tight junction plaque molecules such as zonula occludens-1 and multi-PDZ domain protein-1 in breast cancer are associated with a poor prognosis (33,34). The Wnt signaling pathway is a highly conserved and complicated network, in which the abnormal activation of the canonical Wnt/ β -catenin pathway can lead to the anomalous expression of tumor-associated genes and impact EC progression. Chen *et al* (35) reported that β -catenin and c-myc were activated due to the upregulation of Wnt10b expression, which ultimately promoted the proliferation of Ishikawa cells and inhibited cell apoptosis. Inhibiting the canonical Wnt/ β -catenin pathway or interfering with the regulation of its upstream signals may be a target for anticancer therapy (36-38).

According to the results of the PPI network with DEGs in the present study, the 13 top hub genes are as follows: MAPK1, PHLPP1, ESR1, MDM2, CDKN2A, CDKN1A, AURKA, BCL2L1, POLQ, PIK3R3, RHOQ, EIF4E and LATS2.

The MAPK1 gene, which encodes a member of the MAPK family, is involved in cell proliferation, differentiation and apoptosis. In addition, MAPKs are also known to be extracellular signal-regulated kinases that act as an integration point for biochemical signals. MAPK1 is overexpressed in various solid tumors, such as EC, non-small cell lung cancer, prostate cancer and colorectal cancer (39,40). Wang *et al* (41) demonstrated that insulin receptors are activated by visfatin, which in turn activates the PI3K/Akt and MAPK/ERK signaling pathways. This process is thought to be one of the molecular mechanisms by which visfatin promotes the progression of EC.

PHLPP1 is encoded by a member of the serine/threonine phosphatase family. The function of encoded proteins is to act

Table II. GO analysis of differentially expressed genes associated with patients with different types of endometrial carcinoma.

Category	Term	Count	%	P-value
Upregulated				
GOTERM_BP_FAT	GO:0043065~positive regulation of apoptosis	12	0.8	6.9x10 ⁻⁴
GOTERM_BP_FAT	GO:0043068~positive regulation of programmed cell death	12	0.8	7.3x10 ⁻⁴
GOTERM_BP_FAT	GO:0010942~positive regulation of cell death	12	0.8	7.6x10 ⁻⁴
GOTERM_BP_FAT	GO:0008629~induction of apoptosis by intracellular signals	5	0.3	9.3x10 ⁻⁴
GOTERM_BP_FAT	GO:0006631~fatty acid metabolic process	8	0.5	1.1x10 ⁻³
GOTERM_CC_FAT	GO:0009898~internal side of plasma membrane	7	0.4	4.1x10 ⁻²
GOTERM_MF_FAT	GO:0017137~Rab GTPase binding	3	0.2	2.5x10 ⁻²
GOTERM_MF_FAT	GO:0008565~protein transporter activity	4	0.3	3.4x10 ⁻²
GOTERM_MF_FAT	GO:0003705~RNA polymerase II transcription factor activity, enhancer binding	3	0.2	4.0x10 ⁻²
GOTERM_MF_FAT	GO:0019207~kinase regulator activity	4	0.3	4.0x10 ⁻²
GOTERM_MF_FAT	GO:0003857~3-hydroxyacyl-CoA dehydrogenase activity	2	0.1	4.8x10 ⁻²
Downregulated				
GOTERM_BP_FAT	GO:0006351~transcription, DNA-dependent	7	0.6	1.1x10 ⁻²
GOTERM_BP_FAT	GO:0032774~RNA biosynthetic process	7	0.6	1.2x10 ⁻²
GOTERM_BP_FAT	GO:0006915~apoptosis	10	0.8	1.5x10 ⁻²
GOTERM_BP_FAT	GO:0012501~programmed cell death	10	0.8	1.6x10 ⁻²
GOTERM_BP_FAT	GO:0008637~apoptotic mitochondrial changes	3	0.2	1.7x10 ⁻²
GOTERM_CC_FAT	GO:0005654~nucleoplasm	16	1.3	4.9x10 ⁻⁴
GOTERM_CC_FAT	GO:0070013~intracellular organelle lumen	24	1.9	7.2x10 ⁻⁴
GOTERM_CC_FAT	GO:0031981~nuclear lumen	21	1.7	7.9x10 ⁻⁴
GOTERM_CC_FAT	GO:0044451~nucleoplasm part	12	1.0	8.9x10 ⁻⁴
GOTERM_CC_FAT	GO:0043233~organelle lumen	24	1.9	9.9x10 ⁻⁴
GOTERM_MF_FAT	GO:0000166~nucleotide binding	27	2.2	2.2x10 ⁻³
GOTERM_MF_FAT	GO:0005524~ATP binding	19	1.5	7.3x10 ⁻³
GOTERM_MF_FAT	GO:0032559~adenyl ribonucleotide binding	19	1.5	8.4x10 ⁻³
GOTERM_MF_FAT	GO:0030554~adenyl nucleotide binding	19	1.5	1.4x10 ⁻²
GOTERM_MF_FAT	GO:0032555~purine ribonucleotide binding	21	1.7	1.5x10 ⁻²

GO, Gene Ontology; BP, biological processes; CC, cell component; MF, molecular function.

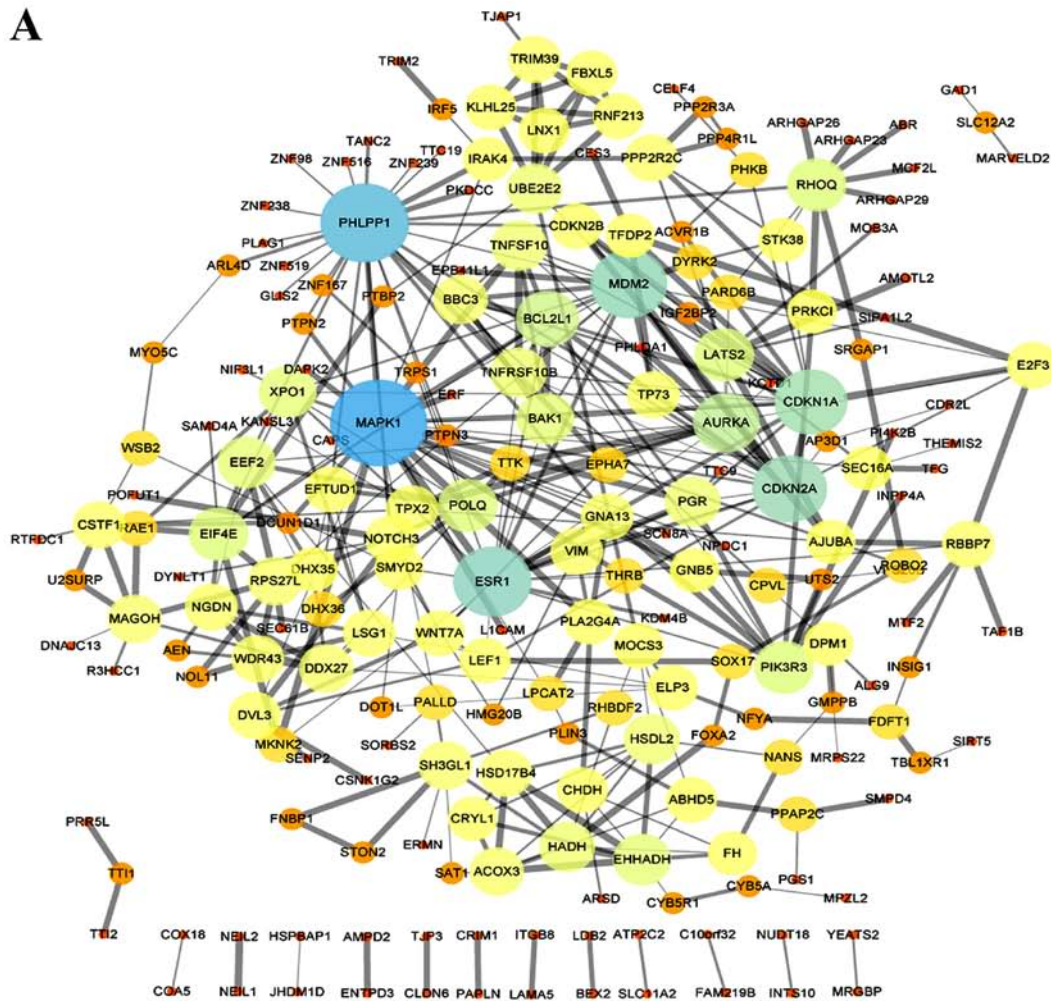
Table III. Kyoto Encyclopedia of Genes and Genomes pathway analysis of differentially expressed genes associated with patients with different types of endometrial carcinoma.

Term	Count	%	P-value	Genes
Upregulated				
hsa04115: p53 signaling pathway	5	0.3	5.6x10 ⁻³	CDKN1A, TNFRSF10B, BBC3, SESN1, TP73
hsa00310: Lysine degradation	3	0.2	3.4x10 ⁻²	AADAT, DOT1L, HADH
Downregulated				
hsa05200: Pathways in cancer	10	0.8	2.7x10 ⁻⁴	DVL3, ACVR1B, E2F3, CDKN2A, CDKN2B, LAMA5, PAX8, TFG, BCL2L1, WNT7A
hsa04530: Tight junction	4	0.3	7.8x10 ⁻³	PAR6B, CLDN6, PRKCI, TJAP1
hsa04110: Cell cycle	5	0.4	1.0x10 ⁻²	E2F3, CDKN2A, CDKN2B, TFDP2, TTK
hsa04310: Wnt signaling pathway	4	0.3	1.1x10 ⁻²	SEN2, DVL3, TBL1XR1, WNT7A
hsa05220: Chronic myeloid leukemia	4	0.3	1.4x10 ⁻²	ACVR1B, E2F3, CDKN2A, BCL2L1
hsa05222: Small cell lung cancer	4	0.3	2.3x10 ⁻²	E2F3, CDKN2B, LAMA5, BCL2L1

as tumor inhibitors in a number of different types of cancer, and their possible molecular mechanism is to regulate apoptosis by

dephosphorylation and inactivate the serine/threonine kinase Akt. Thus, PHLPP1 has an effect on mediating the activation

A



B

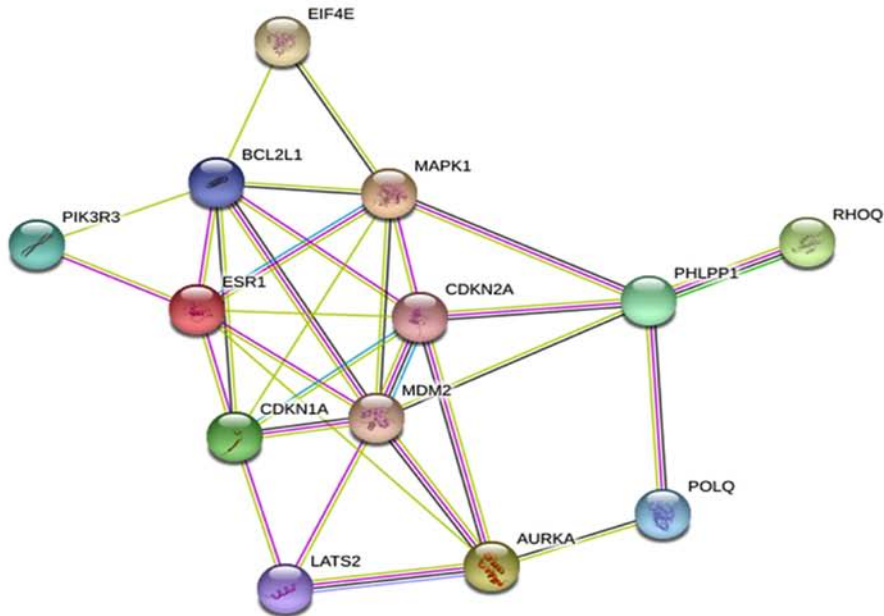


Figure 3. PPI network of the differentially expressed genes and the top 13 hub genes. (A) PPI network and hub genes of the differentially expressed genes. Nodes were given different colors according to the interaction values between two genes. Yellow nodes represent a low value (darker node represents a smaller value) whereas blue nodes represent a high value (darker node represents a higher value). (B) PPI network of the top 13 hub genes. PPI, protein-protein interaction.

of the PI3K/AKT signaling pathway (42). It has been reported that significant downregulation or loss of PHLPP1 is closely associated with metastasis in a variety of different types of

tumor, such as colorectal cancer, breast cancer and gastric cancer (43,44). This finding coincides with the results from the present study, in that PHLPP1 was more significantly

Table IV. Top 13 hub genes of the protein-protein interaction network with higher degrees of connectivity.

Hub gene	Gene name	Node degree
MAPK1	Mitogen-activated protein kinase 1	32
PHLPP1	Ph domain and leucine-rich repeat protein phosphatase 1	27
ESR1	Estrogen receptor 1	21
MDM2	Mdm2 proto-oncogene	20
CDKN2A	Cyclin-dependent kinase inhibitor 2a (Melanoma, p16, Inhibits Cdk4)	19
CDKN1A	Cyclin-dependent kinase inhibitor 1a	18
AURKA	Aurora kinase a	14
BCL2L1	Bcl-2-like 1	12
POLQ	Dna polymerase τ	10
PIK3R3	Phosphoinositide-3-kinase regulatory subunit 3	10
RHOQ	Ras homolog family member q	10
EIF4E	Eukaryotic translation initiation factor 4e	10
LATS2	Large tumor suppressor kinase 2	10

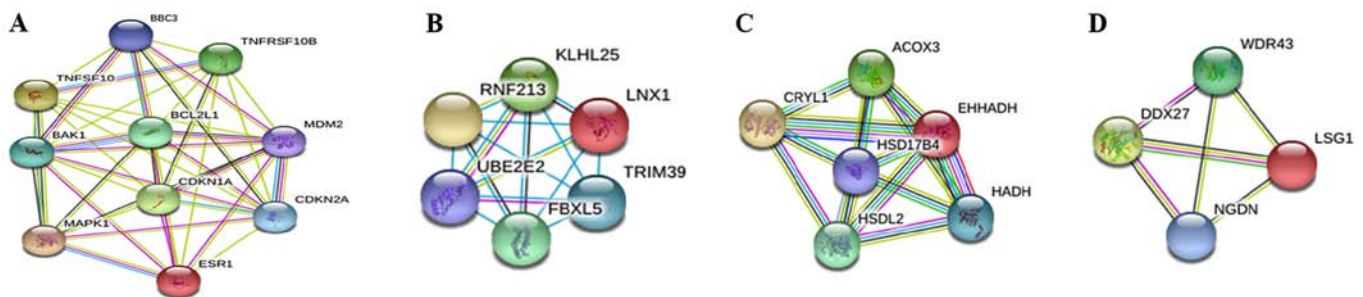


Figure 4. Significant module and enrichment analyses from the protein-protein interaction network. Only modules with a score >4 were presented. (A) Module 1. (B) Module 2. (C) Module 3. (D) Module 4.

downregulated in type II EC than in type I EC. PHLPP1 phosphatases have a clinically relevant role in the pathogenesis of insulin resistance-associated diseases, including EC (45). To the best of our knowledge, there has been no study to date regarding the role of PHLPP1 in EC.

The third hub gene, ESR1, encodes an estrogen receptor and has been noted in several pathological processes of breast cancer and endometrial cancer (46). ESR1 is an estrogen-dependent transcription factor discovered by Jensen in 1971 (47). ESR1 is also a member of the nuclear receptor superfamily, which can bind to estrogen, transfer to the nucleus of breast cancer cells and participate in the regulation of gene transcription signaling pathways, thus promoting the proliferation of breast cancer cells (48). The ESR1 gene has been a focus of breast cancer studies for quite some time, but it is also clinically relevant in endometrial, ovarian and other types of cancer.

The MDM2 gene is an oncogene closely associated with the function of p53. MDM2 has been studied in regard to tumors such as lung cancer and uterine sarcoma (49), but its mechanism in the occurrence and development of EC has rarely been reported. The MDM2 protein is overexpressed in both primary and metastatic EC, which also indicates that MDM2 protein expression is closely associated with the development of EC (50). In addition, Wu and Luo (51) revealed

that the MDM2 rs2279744 polymorphism was significantly associated with EC risk in the Chinese Han population.

CDKN1A is a gene that encodes a potent inhibitor of cyclin. The encoded protein CDKN1A regulates the progression of the cell cycle and focuses on the G₁ phase of cell division, as it directly and indirectly binds to and inhibits the activity of complexes with cyclin-dependent kinase 2 or cyclin-dependent kinase 4 (52). The diseases associated with CDKN1A include bladder cancer (53) and pancreatic inflammation (54). The loss of CDKN2A has been demonstrated to be an important event in various types of tumor. Although no clinical trials have been conducted on targeted therapies as of yet, the impact of CDKN1A on the prognosis and chemotherapy resistance in pancreatic and breast cancer have been studied (55).

A protein that seems to mediate microtubule formation and/or stability at the spindle end during chromosome segregation is encoded by the AURKA gene, which is essentially a protein kinase capable of regulating the self-reporting cycle. Recent evidence indicates that the overexpression of AURKA is associated with high tumor grade, severe histological type and paclitaxel sensitivity in EC (56). Furthermore, previous studies have demonstrated that patients with EC who are resistant to conventional surgical treatment and chemotherapy have short survival

Table V. GO and Kyoto Encyclopedia of Genes and Genomes enrichment analyses of genes in the significant module, with only the top 3 BPs presented.

Category	Term	P-value	Genes
GOTERM_BP_FAT	GO:0043065~positive regulation of apoptosis	1.1x10 ⁻⁹	BAK1, MAPK1, CDKN1A, TNFSF10, CDKN2A, TNFRSF10B, BBC3, BCL2L1
GOTERM_BP_FAT	GO:0043068~positive regulation of programmed cell death	1.1x10 ⁻⁹	BAK1, MAPK1, CDKN1A, TNFSF10, CDKN2A, TNFRSF10B, BBC3, BCL2L1
GOTERM_BP_FAT	GO:0010942~positive regulation of cell death	1.2x10 ⁻⁹	BAK1, MAPK1, CDKN1A, TNFSF10, CDKN2A, TNFRSF10B, BBC3, BCL2L1
KEGG_PATHWAY	hsa04115: p53 signaling pathway	9.9x10 ⁻⁷	CDKN1A, CDKN2A, TNFRSF10B, BBC3, MDM2
KEGG_PATHWAY	hsa05220: Chronic myeloid leukemia	1.5x10 ⁻⁶	MAPK1, CDKN1A, CDKN2A, MDM2, BCL2L1
KEGG_PATHWAY	hsa05219: Bladder cancer	1.8x10 ⁻⁵	MAPK1, CDKN1A, CDKN2A, MDM2,

GO, Gene Ontology; BP, biological process.

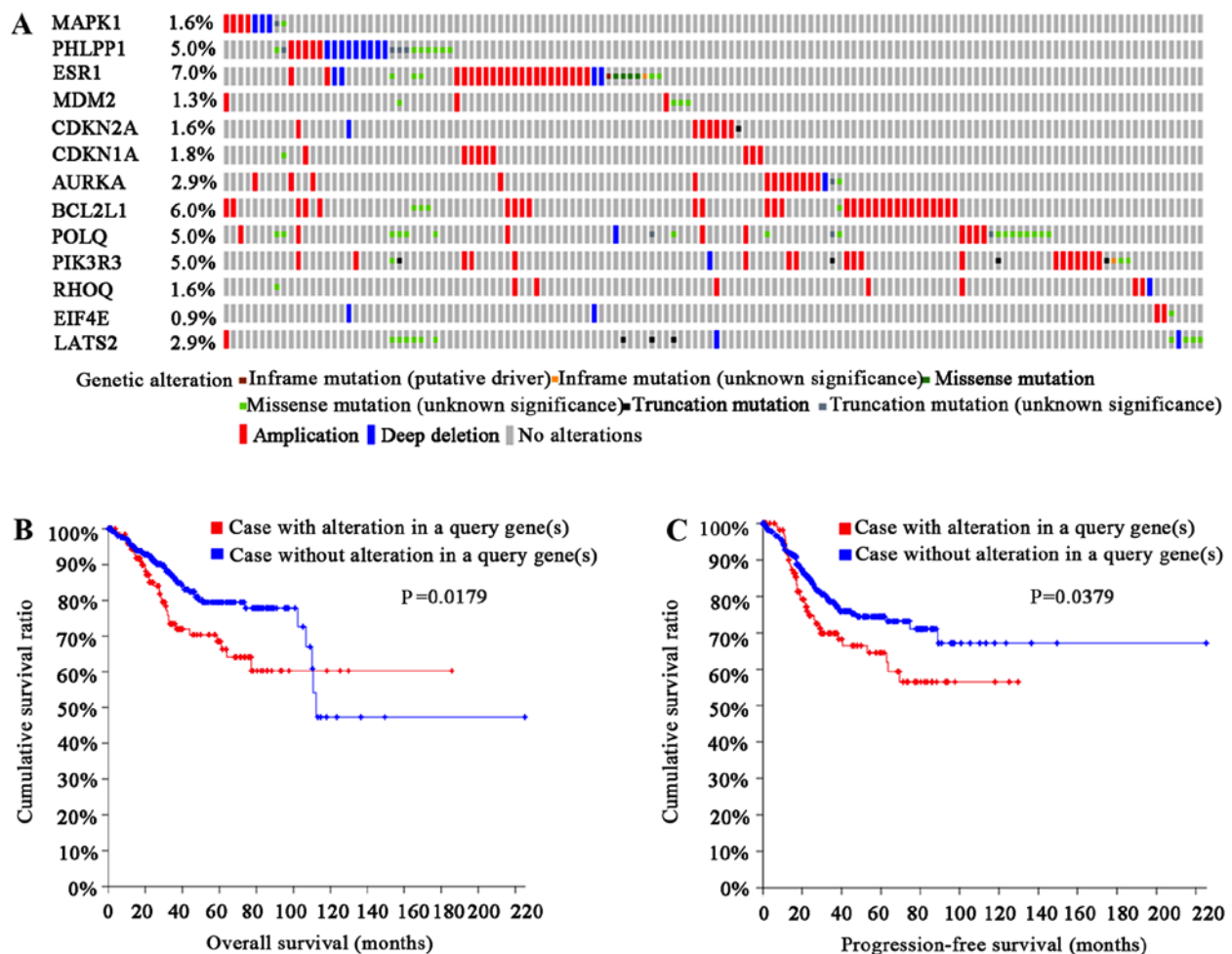


Figure 5. (A) Genetic alterations. Red represents amplification and blue represents deep deletion of genes in 136 of the 547 endometrial carcinoma patients (25%). The aberrant expression threshold was defined as z-score ± 2.0 from The Cancer Genome Atlas RNA Sequencing version 2 data. A Kaplan-Meier curve was created between groups with and without alterations. (B) Overall survival Kaplan-Meier estimate. (C) Progression-free survival Kaplan-Meier estimate. The red and blue lines represent cases with and without alterations, respectively.

times and poor prognosis (57). However, the combination of an AURKA inhibitor and paclitaxel can be effective in these patients with EC, so it is speculated that AURKA may be a promising target in EC therapy (58).

Another hub gene, BCL2L1 is a member of the BCL-2 protein family and forms hetero- or homodimers to act as anti-apoptotic or pro-apoptotic regulators that are involved in programmed cell death or apoptosis activities (59). Studies

Table VI. Results of mutual exclusivity and co-occurrence analysis by cBioPortal.

Gene A	Gene B	P-value	Adjusted P-value	Association
PHLPP1	LATS2	<0.001	0.002	Tendency towards co-occurrence
POLQ	LATS2	<0.001	0.007	Tendency towards co-occurrence
PHLPP1	POLQ	<0.001	0.014	Tendency towards co-occurrence
ESR1	CDKN1A	<0.001	0.014	Tendency towards co-occurrence
PHLPP1	PIK3R3	<0.001	0.030	Tendency towards co-occurrence
POLQ	ESR1	<0.001	0.046	Tendency towards co-occurrence
MDM2	LATS2	<0.001	0.052	Tendency towards co-occurrence
AURKA	BCL2L1	0.002	0.142	Tendency towards co-occurrence
ESR1	LATS2	0.002	0.186	Tendency towards co-occurrence
PHLPP1	BCL2L1	0.003	0.214	Tendency towards co-occurrence

The analysis contained 22 gene pairs with mutually exclusive alterations (none significant) and 56 gene pairs with co-occurring alterations (18 significant and only the top 10 were shown).

have revealed that both lung cancer and pancreatic cancer are associated with BCL2L1. The associated pathways of BCL2L1 include apoptosis regulation, signal transduction and chronic myeloid leukemia progression (60).

PIK3R3 serves as a second messenger in growth signaling pathways; it encodes the regulatory subunit PI3K that can activate (phosphorylate) tyrosine protein kinases via binding to the SH2 domain (61). A previous study (62) revealed that the mRNA expression and the expression of PIK3R3 was markedly increased in ovarian cancer samples compared with that in normal ovarian samples, and the difference was statistically significant. Cellular experiments further demonstrated that the proliferation, migration and invasion of the ovarian cancer SKOV3 cells were regulated by the interaction between HOTAIR and PIK3R3 (63). However, given the limited research available, it is not possible to speculate on the association between PIK3R3 and EC.

Furthermore, the small GTPases that are encoded by RHOQ belong to the ρ family. GTPases alternate between inactive GDP and the active GTP binding state, and act as a molecular switch in the signal transduction cascade. The associated pathways include ERK signaling and Akt signaling. In diffuse subtypes of gastric cancer, the overexpression of the RHOQ gene is correlated with a poor prognosis (64).

One of the proteins that make up the eukaryotic translation initiation factor 4F complex is encoded by the EIF4E gene, which can recognize mRNAs through the structure of the 7-methylguanosine cap at the 5' tail. The overexpression of the EIF4E gene and high expression levels of its proteins were noted in the EC specimens, and were associated with FIGO stage, histological grade and degree of lymphatic metastasis (65). The positive expression rates of eIF4E were not only associated with the EC stages as determined by FIGO stage but were also associated with tumor cell differentiation degree and lymphatic metastasis (66). As noted, EIF4E may serve a crucial role in the pathogenesis of EC and become a potential trigger in carcinogenesis.

LATS2 is a tumor suppressor that encodes the corresponding protein kinase, namely, serine/threonine protein kinase. LATS2 mediates the mutation of normal cells into tumor cells by regulating the cell cycle, proliferation and

apoptosis of tumor cells. LATS2 not only interacts with p53 in a positive feedback loop to participate in the response to cytoskeletal injury, but LATS2 is also regarded as a co-inhibitory factor with androgen. By limiting proliferation and promoting apoptosis, negative regulators of YAP1 involved in the regulation of the Hippo signaling pathway play a crucial role in organ size control and tumor inhibition (67). Zhao *et al* (68) showed that prostatic invasion and metastasis were notably downregulated by LATS2 expression in the LATS2-Yap1 signaling pathway. Notably, in a previous study, LATS2 was confirmed as the target of MIR31 in EC cells, as LATS2 was downregulated among MIR31-overexpressing cells (69). These studies were consistent with the results from the present study, showing that LATS2 was significantly downregulated in type II EC.

Currently, the biological functions of CDKN2A, BCL2L1, POLQ and RHOQ in EC are unknown.

Module analysis of the PPI network revealed that the progression and histological types of EC were positively associated with the regulation of apoptosis and programmed cell death. Regarding histogenesis of these type II tumors, the concept of resting/atrophic endometrial transformation into endometrial intraepithelial carcinoma has been proposed and increasing evidence has demonstrated that p53 features are associated with the dynamic transformation of endometrial carcinoma development (70). Serous carcinoma typically displays significant overexpression of p53 and p16 (71). There was a large difference in the functions of the DEGs between type I and type II endometrial cancer in the present study, which suggested that there were also great differences in the biological characteristics of these two types of endometrial cancer.

In conclusion, by comparing the type I and type II EC samples, a total of 9,671 DEGs, including 5,962 upregulated and 3,709 downregulated DEGs, were identified. The 13 top hub genes, MAPK1, PHLPP1, ESR1, MDM2, CDKN2A, CDKN1A, AURKA, BCL2L1, POLQ, PIK3R3, RHOQ, EIF4E and LATS2, were identified from the PPI network. The key pathways and genes associated with the histological types of EC, endometrioid adenocarcinoma and serous adenocarcinoma, were identified. Based on the enrichment analysis of DEGs, they were primarily associated with the positive

regulation of apoptosis, programmed cell death and cell death. The altered DEGs (MAPK1, MDM2, AURKA, EIF4E and LATS2) may play roles in the tumor differentiation of EC. The present study highlights the novel molecular features of serous adenocarcinoma. These identified key genes may help to identify the potential molecular mechanism underlying tumorigenesis, and additionally serve as candidate biomarkers and potential targeted therapy key points for type II EC. However, the present study had a limitation, as the findings were not validated in clinical samples. More in-depth experimental studies are necessary to sufficiently confirm the role of these DEGs in these two different types of EC.

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Availability of data and materials

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

Authors' contributions

KZ drafted this manuscript. KZ and HL conceived and designed the study. YY, KL and YZ collected the data and performed the analyses. YW and FX helped to design the study and revised the manuscript. All authors approved the final version of manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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