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Integrated analysis of differentially expressed genes implicated in ovarian cancer progression

Diferansiyel olarak ifade edilen genlerin entegre analizi yumurtalık kanserinin ilerlemesinde rol oynar

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Abstract

Objective: Ovarian cancer (OC) is a common gynecological malignancy associated with high morbidity and generally poor prognosis despite treatment. The aim of this study was to understand the influence of gene expression differences and pathways in OC development and progression.

Materials and Methods: One hundred and thirty-three OC samples and 34 normal ovarian tissues were included in the study from the Gene Expression Omnibus database. GeneSpring Software was used to obtain differentially expressed genes (DEGs) in all stages comparing tumor and normaltissue. DEGs were analyzed using the DAVID interface for Kyoto Encyclopedia of Genes and Genomes pathway analysis. Most most connected genes were selected as hub genes for each stage using the STRING application in Cytoscape software.

Results: DEGs were found to be associated with cell cycle and herpes simplex virus infection pathways. A total of 19 genes (*ACTB, AKT1, ALB, CTNNB1, EGFR, EP300, ESR1, FN1, GAPDH, HSPA4, IL6, JUN, MYC, PTEN, RPS27A, SRC, TNF, TP53* and *UBC*) were identified as hub genes. Among the hub genes, the *TP53* gene was found to have the highest level of connections in all stages. EGFR, RPS27A, and AKT1 were found to have high numbers of connections in stages II, III, and IV, respectively.

Conclusion: The results of the current study may provide new insights into OC pathogenesis and suggest potential prognostic and therapeutic targets.

Keywords: Ovarian cancer, gene expression, hub genes, integrated analysis

Öz

Amaç: Yumurtalık kanseri, tedavi çabalarına rağmen yüksek morbiditeye sahip olup ve genellikle kötü prognoz ile ilişkili yaygın bir jinekolojik malignite olarak bilinmektedir. Çalışmamızda, gen ekspresyon farklılıklarının ve moleküler yolakların yumurtalık kanseri gelişimi ve ilerlemesindeki rolünün araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya yüz otuz üç yumurtalık kanseri ve 34 normal yumurtalık dokusu örneği Gene ExpressionOmnibus veri tabanından indirilerek dahil edilmiştir. Tüm evrelerde diferansiyel olarak ifade edilen genleri (DEG'ler) elde etmek için GeneSpring Yazılımı tümör ve normali karşılaştırarak kullanıldı. DEG'ler, Kyoto Genler ve Genomlar Ansiklopedisi yolak analizi için DAVID arayüzü kullanılarak analiz edilmiştir. Her evredeki merkezi genler, Cytoscape yazılımındaki STRING uygulaması kullanılarak en fazla bağlantıya sahip 15 gen şeklinde belirlenmiştir.

Bulgular: Diferansiyel olarak ifade edilen genler, hücre döngüsü ve herpes simpleks virüs enfeksiyonu yolakları ile ilişkili bulunmuştur. Toplam 19 gen (*ACTB, AKT1, ALB, CTNNB1, EGFR, EP300, ESR1, FN1, GAPDH, HSPA4, IL6, JUN, MYC, PTEN, RPS27A, SRC, TNF, TP53* ve *UBC*) merkezi genler olarak saptanmıştır. Merkezi genler arasında *TP53* geninin tüm evrelerde en yüksek düzeyde bağlantıya sahip olduğu bulunmuştur. EGFR, RPS27A ve AKT1'in sırasıyla evre II, evre III ve evre IV'te yüksek sayıda bağlantıya sahip olduğu dikkati çekmiştir.

Sonuç: Bu çalışmanın sonuçları over kanseri patogenezine ilişkin literatüre yeni bilgiler katabilir ve potansiyel prognostik ve terapötik hedefler önerebilir.

Anahtar Kelimeler: Yumurtalık kanseri, gen ekspresyonu, merkezi genler, entegre analiz

PRECIS: Using public gene expression microarray datasets, we have investigated differentially expressed genes and pathways playing a role in the ovarian cancer progression.

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Introduction

Ovarian cancer (OC) is a prevalent gynecological malignancy associated with high morbidity and generally poor prognosis. Although the 5-year survival rate for early-stage OC patients is 93%, the majority of patients (over 80%) are not diagnosed until the tumor progresses to stage III or IV[1]. Metastasis and recurrence (usually associated with increased chemoresistance) are frequent in ovarian cancer[2].

The poor prognosis and high mortality rate can be mainly attributed to the lack of early and effective detection methods. Thus, increased efforts are required to identify and comprehend new biomarkers and distinct targets of ovarian cancer. Illuminating genetic expression differences in ovarian cancers using the microarray method can be used for diagnostic, prognostic, or therapeutic purposes.

The aim of this study was to analyze gene expression differences in OC and to investigate the influence of associated genes and pathways on the development and/or progression of ovarian cancers using gene expression microarray datasets from stages I, II, III, and IV.

Materials and Methods

Gene Expression Microarray Data

The National Center for Biotechnology Information-Gene Expression Omnibus (NCBI-GEO) database is a free and publicly accessible database containing gene profiles. Gene expression profiles were selected from seven microarray datasets (GSE18520, GSE28044, GSE65986, GSE44104, GSE9891, GSE39204, and GSE63885) in the GEO database. The selected gene expression profiles were based on data obtained from human and normal ovarian tissues and the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array).

A total of 133 patient samples (Stage I: 47, Stage II: 21, Stage III: 41, Stage IV: 24) and 34 control samples were included in the study. From the GSE65986 dataset, 55 patients (Stage I: 30, Stage II: 5, Stage III: 11, Stage IV: 9), from the GSE44104 dataset, 60 patients (Stage I: 17, Stage II: 8, Stage III: 30, Stage IV: 5), from the GSE9891 dataset, 5 patients (Stage II), from the GSE39204 dataset, 3 patients (Stage II), from the GSE63885 dataset, 10 patients (Stage IV) were selected. Control samples were chosen from the GSE28044 dataset including 24 "non-malignant" tissue samples, and from the GSE18520 dataset including 10 "normal ovary" tissue samples. Gene expression microarray raw data for the samples described in the datasets were downloaded from the GEO database.

Identification of Differentially Expressed Genes (DEGs)

GeneSpring Software version 14.9_gx_pa was used to obtain DEGs between tumor and normal tissues. Although GeneSpring is not an open source software, it is user-friendly and has a useful interface for the analysis of genomic and omics data, offering multiple analysis and visualization results. During analysis, DEGs were defined using One-Way ANOVA statistical analysis between tumor and normal tissues, with a p-value threshold of <0.05 and a fold change of >2.0. The Benjamini-Hochberg correction method was used to reduce false positives.

Functional Enrichment Analysis of DEGs

In this study, the online tool DAVID (the Database for Annotation, Visualization and Integrated Discovery) was used to perform gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DEGs. GO analysis included biological processes (BP), cellular components, and molecular functions (MF) categories. Pathway analysis is a functional analysis that matches genes to KEGG pathways. The cutoff criterion was set at p<0.05. A different pathway enrichment analysis tool, the g:GOSt (https://biit.cs.ut.ee/gprofiler/gost) embedded with the g:Profiler web server, was used to confirm the KEGG pathways identified for DEGs of each stage.

Construction of a Protein-protein Interaction (PPI) Network

Cytoscape software version 8.3.2, along with the search tool for the retrieval of interacting genes (STRING) application, was used to explore potential relationships between DEGs at different stages. Although Cytoscape requires knowledge for use, it is an open source software that produces networks containing more interactions compared with commercial software. According to the Cytoscape results, the top 15 genes with high connectivity were selected as hub genes based on PPI information. Hub genes were added to STRING, and GO and KEGG pathway analyses were conducted using DAVID to determine potential information. Another pathway enrichment analysis tool, g:Profiler g:GOSt (https://biit.cs.ut.ee/gprofiler/gost), was used to confirm the KEGG pathways identified for hub genes of each stage.

Analysis of Hub Gene Survival

Overall survival analysis was performed using the ene Expression Profiling Interactive Analysis) (http://gepia.cancer-pku.cn/detail.php), a web tool based on the Cancer Genome Atlas and Genotype-Tissue Expression) gene expression datasets[3]. Default settings, as the cut-off value set to median =50%, hazard ratio (HR) with 95% confidence intervals, and Logrank p-value were used in the single gene analysis module for every hub gene. HR information and log-rank p-values were displayed in the survival plots.

Results

Identifying DEGs

Genes that were expressed differently were defined by comparing the expression rate changes of samples taken from tumor tissues with those of normal tissues using a threshold value of >2.0 in GeneSpring. In stages I, II, III, and IV, 4836, 4249, 4702, and 4340 upregulated genes and 3830, 3421, 3533, and 3848 downregulated genes were identified, respectively.

Pathway Analysis of the DEGs

The most significant molecular pathways defined through pathway analysis of DEGs in tumor tissues using DAVID were the cell cycle for upregulated DEGs and herpes simplex virus infection for downregulated DEGs in stages I, II, III, and I. These pathways were confirmed using g:Profiler and g:GOS. Cell cycle was the most enriched pathway with p-values $5.469x10^{-13}$, $1.935x10^{-10}$, $8.087x10^{-14}$ and $9.804x10^{-11}$ in stages I, II, III, and IV, respectively, according to g:Profiler g:GOSt. Herpes simplex virus infection was the most enriched pathway with p-values $8.335x10^{-8}$, $1.547x10^{-7}$, $2.425x10^{-5}$ and $5.456x10^{-7}$ in stages I, II, III, and IV, respectively, according to g:Profiler g:GOSt. The top five enriched KEGG pathways for upregulated and downregulated DEGs of each stage according to DAVID are presented in Table 1.

GO Analysis of the DEGs

The BP, cellular components, and MF of overexpressed and underexpress genes were determined by GO analysis. Across all stages, upregulated DEGs were most closely associated with cell division in terms of BP, the cytosol in terms of cellular components, and protein binding in terms of molecular functions. Downregulated DEGs were closely related to cilium morphogenesis in terms of BP, cytoplasm in terms of cellular components, and metal ion binding in terms of MF Table 1. However, in stage IV, downregulated DEGs were more closely associated with the cellular component nucleus than with the cytoplasm.

PPI Network

Based on information obtained from publicly available databases such as STRING, PPI networks were constructed for DEGs in each class, and the top 15 genes with the highest level of connections were defined as hub genes Table 2.

KEGG and GO Analysis of Hub Genes

KEGG pathway analysis and GO analysis were performed for hub genes at each stage. According to KEGG pathway analysis using DAVID, hub genes were associated with Kaposi's sarcoma-associated herpesvirus infection in stages I and III and proteoglycans (PGs) in cancer in stages II and IV (Table 3). These pathways were confirmed using g:Profiler and g:GOS. According to pathway enrichment analysis using g:Profiler g:GOSt, Kaposi's sarcoma-associated herpesvirus infection pathway was enriched with a p-value $3.155x10^{-8}$ both in stages I and III. PGs in the cancer pathway were enriched with p-values $9.406x10^{-10}$ and $1.386x10^{-11}$ in stages II and IV, respectively, according to g:Profiler g:GOSt. GO analysis revealed that hub genes were generally associated with positive regulation of transcription DNA-templated in terms of BP, protein-containing complex in terms of cellular components, and enzyme binding in terms of MF Table 4.

Survival Analysis of Hub Genes

The overall survival analysis of 19 hub genes was performed using GEPIA with the default settings (the cut-off value set to median =50%, HRs with 95% confidence intervals). Considering the survival plots, HRs >1 were associated with worse overall survival. Among the hub genes, *MYC* (HR=1.3), epithelial growth factor receptor (*EGFR*) (HR=1.1), *EP300* (HR=1.1), *ESR1* (HR=1.1), *GAPDH* (HR=1.1), *IL6* (HR=1.1), *JUN* (HR=1.1), and *UBC* (HR=1.1) expression were found to be associated with worse overall survival for OC (Figure 1).

Discussion

Although early-stage OC exhibits a 5-year survival rate of approximately 93%, diagnosis is often delayed until Stage III or IV in over 80% of cases, contributing to its overall poor prognosis and high mortality. Therefore, the identification of new biomarkers is crucial for the early detection and development of novel treatment approaches. In alignment with the aim of this study, we obtained datasets from the GEO database to compare 133 OC samples with 34 normal tissue samples.

DEGs were analyzed for KEGG pathways using the DAVID Bioinformatics Database. Upregulated DEGs were primarily associated with the cell cycle, whereas downregulated DEGs were notably associated with herpes simplex virus 1 infection.

Dysregulation of the cell cycle is a hallmark of many cancers, including ovarian cancer. Control and timing of the cell cycle involve checkpoints and regulatory pathways that ensure the accuracy of DNA replication and chromosome segregation. These processes encompass candidate molecules for genetic variants that predispose patients to OC risk. Molecules crucial to the cell cycle, such as *CDK*, *CCNE*, and *E2F*, are overexpressed in various cancers, including ovarian cancer[4]. Studies conducted on OC samples reveal alterations in cell cycle phases, particularly in the G2 phase. Our findings, correlated with literature information, support the suggestion that cell cycle abnormalities in OC may be influenced by genetic variations in genes.

The most common manifestation of herpes simplex virus-1 infection is cold sores on the lips. However, some studies have indicated HSV-1 in various tumor cells. Recently identified herpes virus-associated growth factors with both transforming and transformation-suppressing activities are considered to be significant factors in tumor formation. Furthermore, in two cancer cases, serous ovarian carcinoma and certain prostate tumors, virus-encoded microRNAs were identified as potential cofactors in tumor formation[5]. Further research is needed to understand the mechanisms involved and potential therapeutic interventions.

In our study, 19 genes (*ACTB*, *AKT1*, *ALB*, *CTNNB1*, *EGFR*, *EP300*, *ESR1*, *FN1*, *GAPDH*, *HSPA4*, *IL6*, *JUN*, *MYC*, *PTEN*, *RPS27A*, *SRC*, *TNF*, *TP53*, and *UBC*) were identified as hub genes, with the top 15 genes having the most connections at

each stage. Among the hub genes, the *TP53* gene was found to have the highest level of connections in all stages. *EGFR*, *RPS27A,* and *AKT1* were found to have high numbers of connections in stages II, III, and IV, respectively.

The *TP53* protein is extensively studied and is best known as a DNA-binding transcription factor that can bind to hundreds of different promoter elements in the genome. This characteristic allows it to regulate the expression of numerous genes. Years

Table 1. KEGG pathway and gene ontology analysis of differentially expressed genes associated with ovarian cancer using DAVID

Stage I	Upregulated	**Pathways**	**Biological process**	**Cellular component**	**Molecular function**
		Cell cycle	Cell division	Cytosol	Protein binding
		Cellular senescence	Intracellular protein transport	Membrane	RNA binding
		Parkinson disease	Angiogenesis	Extracellular exosome	Cadherin binding
		Protein processing in the endoplasmic reticulum	Proteasome-mediated ubiquitin	Nucleoplasm	Identical protein binding
		p53 signaling pathway	Mitochondrial translation	Cytoplasm	Ubiquitin protein ligase binding
	Downregulated	Herpes simplex virus 1 infection	Cilium assembly	Cytoplasm	Metal ion binding
		AMPK signaling pathway	Regulation of transcription, DNA-templated	Nucleus	Protein binding
		Autophagy: Animal	Cilium movement	Nucleoplasm	Guanyl-nucleotide exchange factor activity
		FoxO signaling pathway	Cilia-dependent cell motility	Cytosol	Zinc ion binding
		Choline metabolism in cancer	Intracellular signal transduction	Axoneme	Protein serine/threonine kinase activity
Stage II	Upregulated	**Pathways**	**Biological process**	**Cellular component**	**Molecular function**
		Cell cycle	Cell division	Cytosol	Protein binding
		Epstein– Barr virus infection	Angiogenesis	Extracellular exosome	RNA binding
		Phagosome	Positive regulation of cell migration	Membrane	Cadherin binding
		p53 signaling pathway	Apoptotic process	Nucleoplasm	Identical protein binding
		Human T-cell leukemia virus 1 infection	Negative regulation of the apoptotic process	Cytoplasm	Ubiquitin protein ligase binding
	Downregulated	Herpes simplex virus 1 infection	Cilium assembly	Cytoplasm	Metal ion binding
		Autophagy: animal	Regulation of transcription, DNA-templated	Nucleus	Protein binding
		Choline metabolism in cancer	Cilium movement	Axoneme	ATP-dependent microtubule motor activity
		One carbon pool formed by folate	Regulation of transcription from RNA	Cytosol	Zinc ion binding
		SNARE interactions during vesicular transport	Cilia-dependent cell motility	Nucleoplasm	Protein serine/threonine kinase activity
		Pathways	Biological process	Cellular component	Molecular function
Stage III	Upregulated	**Cell cycle**	**Cell division**	**Cytosol**	**Protein binding**
		Prion disease	Angiogenesis	Nucleoplasm	RNA binding
		Parkinson disease	Protein catabolic process	Membrane	Cadherin binding
		Cellular senescence	Mitochondrial translation	Extracellular exosome	Identical protein binding
		Non-alcoholic fatty liver disease	Apoptotic process	Nucleus	Ubiquitin protein ligase binding

Table 1. Continued

Stage III	Downregulated	Herpes simplex virus 1 infection	Cilium assembly	Cytoplasm	Metal ion binding
		FoxO signaling pathway	Regulation of transcription, DNA-templated	Nucleus	Protein binding
		AMPK signaling pathway	Cilium movement	Cytosol	Zinc ion binding
		Autophagy: animal	Negative regulation of transcription	Axoneme	Guanyl-nucleotide exchange factor activity
		Choline metabolism in cancer	Intracellular signal transduction	Motile cilium	Protein serine/threonine kinase activity
		Pathways	**Biological process**	**Cellular component**	**Molecular function**
Stage IV	Upregulated	Cell cycle	Cell division	Cytosol	Protein binding
		Epstein– Barr virus infection	Angiogenesis	Membrane	Cadherin binding
		Human T-cell leukemia virus 1 infection	Positive regulation of transcription	Extracellular exosome	Identical protein binding
		Human papillomavirus infection	Apoptotic process	Nucleoplasm	RNA binding
		Cellular senescence	Cell migration	Cytoplasm	Ubiquitin protein ligase binding
	Downregulated	Herpes simplex virus 1 infection	Cilium assembly	Nucleus	Metal ion binding
		AMPK signaling pathway	Cilium movement	Cytoplasm	Protein binding
		FoxO signaling pathway	Regulation of transcription, DNA-templated	Cytosol	Zinc ion binding
		Autophagy: animal	Regulation of transcription from RNA	Axoneme	Guanyl-nucleotide exchange factor activity
		Antifolate resistance	Negative regulation of transcription	Nucleoplasm	ATP binding

of research on *TP53* have documented its fundamental role in controlling cell proliferation and regulating essential cellular processes that maintain genome integrity and stability[6]. The *TP53* protein responds to various stress signals such as DNA damage, hyperproliferative signals, hypoxia, oxidative stress, and ribonucleotide depletion. Upon activation, it primarily halts the cell cycle, triggers DNA repair, and initiates apoptosis. This leads to the suppression of cellular transformation and proliferation. Over the years, research has revealed TP53's involvement in other cellular processes such as metabolism, angiogenesis, immune responses, stem cell maintenance, and tumor-stromal cell crosstalk[7]. In all ovarian cancers, a significantly high mutation frequency ranging from 50% to 100% has been reported[8]. Moreover, studies have confirmed the overexpression of *TP53* in ovarian cancers, but its prognostic significance remains controversial[9-15]. In our study, the identification of *TP53* as the hub gene with the most connections across all stages and its detection as overexpressed are correlated with previous research findings.

The *EGFR* plays important roles in tumor initiation, angiogenesis, and metastasis[16]. Deregulation of *EGFR* has been reported in several malignancies as well as in ovarian cancer. EGFR expression has been detected in up to 90% of certain histotypes of ovarian tumors[17]. Previous investigations on OC have shown that the EGFR protein is overexpressed in 9-62% of cases and is associated with poor prognosis and decreased therapeutic responsiveness[18]. In patients with pancreatic tumors, specific histotypes of ovarian tumors, and lung cancer patients with EGFR mutations, EGFR inhibitors have been recommended as first-line treatment[16]. In our study, EGFR was found to have a high number of connections in stage II. We suggest that EGFR could be a potential biomarker for the diagnosis and prognosis of ovarian cancer.

Ribosomal protein S27A *(RPS27A)* encodes a ribosomal 40S subunit ribosomal protein. RPS27A is involved in ubiquitin production, regulating cell cycle progression, DNA repair, promoting proliferation, and inhibiting apoptosis. Furthermore, RPS27A is a direct transcriptional factor of p53 and is overexpressed in various organ malignancies such as kidney, breast, colon, lung, liver, brain, thymus, and cervix as well as in leukemia and is associated with poor prognosis[19]. RPS27A has been used as a prognostic biomarker in hepatocellular carcinoma and has been identified as a hub gene with increased expression in OC before[20]. In our study, *RPS27A* was found to be a hub gene in stage III cancer, suggesting the importance of RPS27A in tumorigenesis and OC.

Table 2. List of hub genes according to the stages

Stage I			Stage II			Stage III			Stage IV		
Gene	CD	EL	Gen	CD	EL	Gen	CD	EL	Gen	CD	EL
TP53	1070	↑	*TP53*	986	↑	*TP53*	1063	↑	*TP53*	1005	↑
ACTB	934	↑	*ACTB*	881	↑	*ACTB*	926	↑	*ACTB*	895	↑
GAPDH	874	↑	*GAPDH*	816	↑	*GAPDH*	868	↑	*AKT1*	862	↑
MYC	846	↑	*MYC*	778	↑	*MYC*	839	↑	*GAPDH*	824	↑
CTNNB1	829	↑	*CTNNB1*	755	↑	*CTNNB1*	800	↑	*CTNNB1*	797	↑
SRC	688	↑	*EGFR*	671	↑	*RPS27A*	706	↑	*MYC*	789	↑
UBC	632	↑	*SRC*	632	↑	*SRC*	663	↑	*EGFR*	708	↑
TNF	595	↓	*TNF*	561	↑	*TNF*	572	↑	*SRC*	655	↑
ALB	580	↓	*ALB*	538	↓	*PTEN*	562	↓	*TNF*	588	↑
PTEN	573	↓	*JUN*	513	↑	*ALB*	552	↓	*ALB*	556	↓
ESR1	554	↓	*FN1*	510	↑	*JUN*	546	↑	*PTEN*	544	↓
EP300	551	↓	*IL6*	510	↑	*HSPA4*	542	↑	*IL6*	529	↑
JUN	547	↑	*HSPA4*	506	↑	*EP300*	542	↓	*FN1*	523	↑
IL6	544	↑	*ESR1*	490	↓	*IL6*	531	↑	*EP300*	522	↓
FN1	536	↑	*EP300*	489	↓	*ESR1*	526	↓	*ESR1*	502	↓

CD: Connection degree, EL: Gene expression level, ↑: Up-regulated, ↓: Down-regulated

Table 3. KEGG pathway analysis of hub genes according to the stages using DAVID

	Pathways	p-value
Stage I	Kaposi's sarcoma-associated herpesvirus infection	8,90E-09
	Proteoglycans in cancer	1,30E-08
	Thyroid hormone signaling pathway	2,10E-08
	Hepatitis B	1,20E-07
	Pathways in cancer	4,90E-07
Stage II	Proteoglycans in cancer	2,70E-10
	Thyroid hormone signaling pathway	2,10E-08
	Hepatitis B	1,20E-07
	Kaposi's sarcoma-associated herpesvirus infection	3,50E-07
	Pathways in cancer	4,90E-07
Stage III	Kaposi's sarcoma-associated herpesvirus infection	8,90E-09
	Thyroid hormone signaling pathway	2,10E-08
	Hepatitis B	1,20E-07
	Proteoglycans in cancer	4,80E-07
	Human T-cell leukemia virus 1 infection	7,70E-07
Stage IV	Proteoglycans in cancer	4,30E-12
	Thyroid hormone signaling pathway	3,20E-10
	Pathways in cancer	2,10E-08
	Endometrial cancer	2,40E-08
	Human cytomegalovirus infection	2,50E-08

AKT1 is a member of the AKT serine/threonine protein kinase family that regulates various functions such as cell proliferation, survival, and metabolism. AKT is a key component of signaling pathways and is effective in both normal and malignant cells. *AKT1-3* are overexpressed in ovarian cancer. AKT activation is commonly observed in high-grade serous ovarian cancer[21]. It has been proposed that AKT1 is the main isoform responsible for OC cell proliferation and protection against apoptosis, playing a significant role in OC cell viability[22]. In our study, *AKT1* was identified as one of the hub genes with the most connections in high-grade OC (Stage IV). This finding correlates with the literature and emphasizes that the overexpression may play a role in mediating the progression and metastasis of ovarian tumors.

According to the KEGG pathway analysis, when we look at the top 5 pathways most associated with Hub genes, we observe the PGs in cancer pathway in stages II and IV. In Stage IV, the pathways of endometrial cancer and human cytomegalovirus (HCMV) infection were found to be more significant than those in other stages. PGs are characterized by the covalent attachment of a specialized linear carbohydrate chain composed of repeating disaccharide units called glycosaminoglycans (GAGs). GAG types found in PGs include heparan sulfate and chondroitin sulfate. PGs play essential roles within cells and basal membranes as secreted components of the interstitial extracellular matrix (ECM). In particular, cell surface PGs serve as integral parts of signaling events, modulation of inflammation, and adhesion in the context of tumor formation.

Table 4. Gene ontology analysis of hub genes according to the stages

	Biological process	Cellular component	Molecular function
Stage I	Positive regulation of transcription, DNA-templated	Protein-containing complex	Enzyme binding
	Positive regulation of sequence-specific DNA binding transcription factor activity	Transcription regulator complex	Disordered domain-specific binding
	Negative regulation of the apoptotic process	Nucleoplasm	Transcriptional coregulator binding
	Response to a xenobiotic stimulus	Chromatin	İdentical protein binding
	Positive regulation of the apoptotic process	Euchromatin	Protease binding
Stage II	Positive regulation of transcription, DNA-templated	Protein-containing complex	Enzyme binding
	Negative regulation of the apoptotic process	Transcription regulator complex	Disordered domain-specific binding
	Positive regulation of miRNA transcription	Chromatin	Transcriptional coregulator binding
	Positive regulation of fibroblast proliferation	Euchromatin	Identical protein binding
	Positive regulation of transcription by the RNA polymerase II promoter	Nucleus	Chromatin binding
Stage III	Positive regulation of transcription, DNA-templated	Protein-containing complex	Transcriptional coregulator binding
	Positive regulation of sequence-specific DNA binding transcription factor activity	Transcription regulator complex	Enzyme binding
	Negative regulation of the apoptotic process	Nucleus	Identical protein binding
	Response to a xenobiotic stimulus	Nucleoplasm	RNA polymerase II-specific DNA-binding transcription factor binding
	Positive regulation of the apoptotic process	Chromatin	Chromatin binding
Stage IV	Positive regulation of transcription, DNA-templated	Protein-containing complex	Enzyme binding
	Negative regulation of the apoptotic process	Transcription regulator complex	Identical protein binding
	Positive regulation of sequence-specific DNA binding transcription factor activity	Cytoplasm	Transcriptional coregulator binding
	Positive regulation of transcription by the RNA polymerase II promoter	Nucleus	Disordered domain-specific binding
	Positive regulation of gene expression	Nucleoplasm	Nitric oxide synthase regulator activity

They regulate cell-cell and cell-ECM interactions, affecting processes such as differentiation, proliferation, adhesion, and migration. Alterations in PG expression within tumor cells and the tumor microenvironment are associated with cancer progression[23]. Compared with ovarian tumors, a wider variety of heparan sulfate PGs has been found in normal ovaries. In addition, a specific type of heparan sulfate PG, syndecan-1, has been proposed to contribute to stromal induction in the pathogenesis of ovarian malignancies[24]. Understanding these changes could lead to the development of diagnostic biomarkers and more targeted therapies.

Endometrial cancer is a commonly occurring type of female reproductive system cancer originating from the lining of the uterus and is often diagnosed post-menopause. Cancer is classified into two distinct types based on biological characteristics and clinical behavior. Type I carcinoma is associated with heightened estrogen levels and is often linked to endometrial hyperplasia. It frequently displays estrogen and progesterone receptors and occurs in younger age groups. On the other hand, type II carcinoma is not linked to estrogen, often arises in the atrophic endometrium, lacks estrogen and progesterone receptors, and typically affects older individuals. Morphological disparities between these cancer types are mirrored in their molecular genetic profiles. Type I is marked by DNA mismatch repair defects and mutations in the ***PTEN***, ***K-ras***, and ***beta-catenin*** genes. In contrast, type II anemia, ***TP53*** gene mutations, and her2/neu amplification[25]. Factors such as similar histological subtypes and gene expression profiles between endometrial and ovarian cancers indicate commonalities between these two types of cancer[26]. Moreover, it has been reported that the two cancers can occur concurrently as independent tumors or metastatic tumors[27,28]. The ***PTEN***, ***beta-catenin*** (***CTNNB1***), and ***TP53*** genes highlighted in endometrial cancers were also identified as hub genes in all stages of our study. Our findings reinforce the similarities between the two cancers, and the closer relationship in Stage IV cancer suggests a potential consideration of synchronous endometrial metastasis risk.

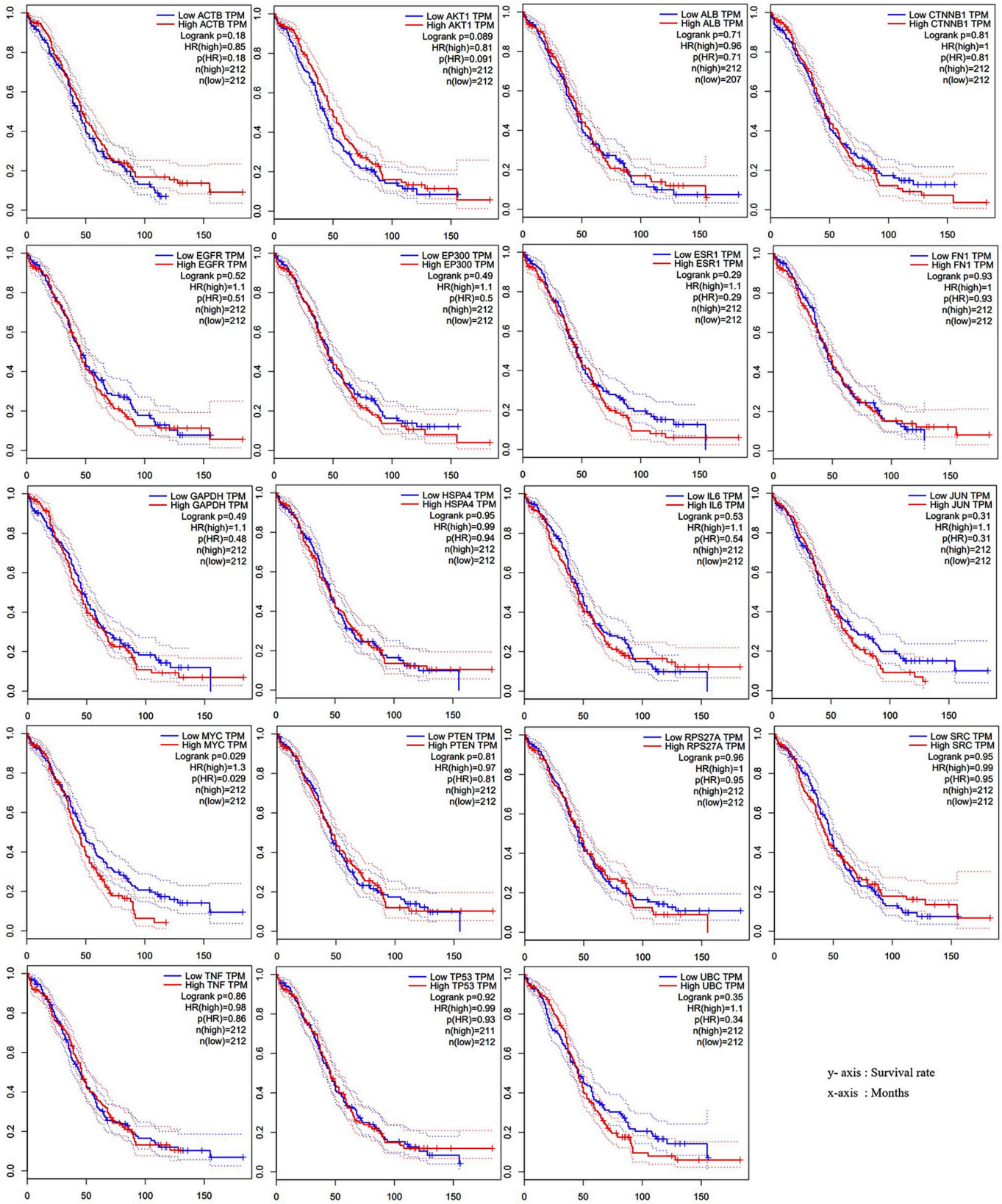

Figure 1. Survival graphs of hub genes in ovarian cancer

HR: Hazard ratio

HCMV produces approximately 200 proteins, 50 of which are crucial for replication. New ribosome profiling data suggest that over 750 unique RNA code for viral proteins. Many of these factors affect cellular and immunological functions relevant to tumor development. Recent studies indicate that the oncomodulatory properties of HCMV are important in carcinogenesis; its proteins interact with key cellular factors and pathways[29]. HCMV blocks apoptosis and evades immune surveillance, giving infected cells a survival advantage[30]. It also alters the expression of matrix metalloproteinases associated with aggressive tumors[31]. Shanmughapriya et al.[32] detected HCMV-glycoprotein B DNA in approximately 50% of OC tissues using the polymerase chain reaction. This suggests that HCMV infection in the tumor microenvironment could support cancer progression or metastasis. Intense HCMV expression is linked to shorter survival in patients with ovarian cancer, whereas higher HCMV IgG levels are associated with better prognosis[33]. The increased association of HCMV with stage IV cancer supports its link with poor prognosis. A better understanding of the oncomodulatory and immunomodulatory roles of HCMV in OC is needed. Therefore, immunotherapies could be potential targets for advanced treatment strategies in ovarian cancer.

Study Limitations

This study has limitations because of the limited sample size derived from microarray datasets and the absence of survival analysis on sufficient clinical samples. In future prospective studies with larger sample sizes, assessing the clinical significance of hub genes identified as biomarkers for ovarian cancers is crucial.

Conclusion

In conclusion, this study identified DEGs between ovarian cancers and normal ovarian tissues by analyzing seven gene expression microarray datasets. *ACTB*, *AKT1*, *ALB*, *CTNNB1*, *EGFR*, *EP300*, *ESR1*, *FN1*, *GAPDH*, *HSPA4*, *IL6*, *JUN*, *MYC*, *PTEN*, *RPS27A*, *SRC*, *TNF*, *TP53,* and *UBC* were identified as hub genes in our study. Among these hub genes, the *TP53* gene was found to have the most interactions in all stages, suggesting that *TP53* may contribute to OC development. *EGFR* was found to have the highest interactions in stage II. We suggest that EGFR is a potential biomarker for the prognosis of ovarian cancer. In our study, *RPS27A* was found to be a hub gene in stage III, suggesting the importance of *RPS27A* in tumorigenesis and OC progression. *AKT1* was identified as a hub gene with the highest number of interactions in high-grade OC (Stage IV). This finding emphasizes that the overexpression of *AKT1* may mediate the progression and metastasis of ovarian tumors. The findings of this study are expected to shed light on the development, progression, and differentiation of ovarian cancers and contribute to the development of novel therapeutic approaches through new clinical, epidemiological, and experimental studies.

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Ethics

Ethics Committee Approval: Our research is based on open-source data and therefore does not require ethics committee approval.
Informed Consent: Informed consent was obtained from all participants.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: B.G., D.S.A., Design: B.G., D.S.A., Data Collection or Processing: B.G., N.K.S., Analysis or Interpretation: B.G., N.K.S., D.S.A., Literature Search: B.G., N.K.S., D.S.A., Writing: B.G., N.K.S., D.S.A.
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