A gene interaction network-based method to measure the common and heterogeneous mechanisms of gynecological cancer

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Abstract. Gynecological malignancies are a leading cause of mortality in the female population. The present study intended to identify the association between three severe types of gynecological cancer, specifically ovarian cancer, cervical cancer and endometrial cancer, and to identify the connective driver genes, microRNAs (miRNAs) and biological processes associated with these types of gynecological cancer. In the present study, individual driver genes for each type of cancer were identified using integrated analysis of multiple microarray data. Gene Ontology (GO) has been used widely in functional annotation and enrichment analysis. In the present study, GO enrichment analysis revealed a number of common biological processes involved in gynecological cancer, including 'cell cycle' and 'regulation of macromolecule metabolism'. Kyoto Encyclopedia of Genes and Genomes pathway analysis is a resource for understanding the high-level functions and utilities of a biological system from molecular-level information. In the present study, the most common pathway was 'cell cycle'. A protein-protein interaction network was constructed to identify a hub of connective genes, including minichromosome maintenance complex component 2 (MCM2), matrix metalloproteinase 2 (MMP2), collagen type I α1 chain (COL1A1) and Jun proto-oncogene AP-1 transcription factor subunit (JUN). Survival analysis revealed that the expression of MCM2, MMP2, COL1A1 and JUN was associated with the prognosis of the aforementioned gynecological cancer types. By constructing an miRNA-driver gene network, let-7 targeted the majority of the driver genes. In conclusion, the present study demonstrated a connection model across three types of gynecological cancer, which was useful in identifying potential diagnostic markers and novel therapeutic targets, in addition to in aiding the prediction of the development of cancer as it progresses.

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Abbreviations: miRNAs, microRNAs; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, Protein-protein Interaction; DEGs, differentially expressed genes; GEO, Gene Expression Omnibus; MERAV, Metabolic Gene Rapid Visualizer Database; HPRD, Human Protein Reference Database; MMPs, matrix metalloproteinases; ECM, extracellular matrix

Key words: gynecological cancer, driver gene, connection model, bioinformatics

Introduction

Gynecological malignancies, particularly ovarian cancer, cervical cancer and endometrial cancer, are serious medical conditions in women and have been leading causes of cancer mortality in recent years. However, the use of cancer markers for early and progressive detection remain lacking (1). In addition, research has demonstrated that there are close associations across the three aforementioned types of cancer. It has been demonstrated that the progress and the development of the three aforementioned types of cancer are similar, which may be useful when diagnosing any one of these three cancer types. In the case of endometrial cancer, prior to the development of endometrial carcinoma, the endometrium undergoes progressive neoplastic alterations in a parallel fashion to the premalignant alterations observed in the cervix prior to the development of cervical carcinoma (2). The rationale of oophorectomy in surgical management is that endometrial cancer may metastasize to the ovary, in which women with endometrial cancer are at risk for synchronous and metachronous ovarian cancer, and the source of estrogen may be eliminated by oophorectomy (3,4). In cancer cells, oncogenic transformation is associated with major alterations in gene expression (5). With the advent of large-scale screening of cancer genomes, hundreds of genes with alterations in different types of tumors from patients with cancer have been identified (6-10), which revealed that cancer is a complex disease caused by genetic alterations in multiple genes (11,12). In order to elucidate the cancer marker genes and biological processes associated with each type of gynecological tumor, and the potential underlying mechanism of associations among gynecological tumors, the contribution of identified differentially expressed genes (DEGs) to the pathogenesis of gynecological tumors must be understood.

To analyze different DEGs, high-throughput experimental methods, including microarray analysis, have been widely used in a number of studies (13,14). A vast quantity of microarray data has been produced and deposited in publicly-available data repositories, including the Gene Expression Omnibus (GEO) (15). With the methods of integrated bioinformatics

analysis, researchers have been able to advance the identification of genetic signatures. This may provide insights into the underlying biological mechanisms of the development of gynecological tumors.

Chung *et al* (16) revealed that microRNA (miRNA)-200b/a is a direct transcriptional target of grainyhead like transcription factor 2, which is associated with development and overall survival in epithelial ovarian cancer. Halabi *et al* (17) demonstrated that 41 genes, including matrix metalloproteinase (MMP)7 and tumor protein 53, were involved in the potential underlying mechanisms of ovarian cancer. Espinosa *et al* (18) revealed that six genes encoding cyclin B2, cell-division cycle protein 20, protein regulator of cytokinesis 1, synaptonemal complex protein 2, nucleolar and spindle associated protein 1 and cyclin-dependent kinase inhibitor 2 belonging to the mitosis pathway, were potential markers for screening or therapeutic targets of cervical cancer. However, biomarkers which were identified in this way have had poor translation into actual clinical practices. Results have been non-concordant among studies due to small sample sizes. In addition, the studies into the associations of biomarker genes (driver genes) remain lacking among the different types of gynecological tumors.

A robust driver gene biomarker signature may be beneficial for the diagnosis and targeted treatment of gynecological tumors. In the present study, in order to identify a driver gene biomarker signature for the three types of gynecological tumors, data from the Metabolic Gene Rapid Visualizer database (MERAV, which is derived from GEO) was used (19). In MERAV, microarrays were normalized together to eliminate systematic errors caused by different batch experiments.

The present study devised a target network for ovarian cancer, cervical cancer and endometrial cancer using the selected driver genes, and further investigated the identified DEGs via functional enrichment analysis, pathway enrichment analysis and protein-protein interaction (PPI) networks. In addition, the present study extracted clinical information of ovarian cancer, cervical cancer and endometrial cancer from The Cancer Genome Atlas (TCGA) data portal. Subsequently, driver genes in each type of cancer were analyzed. It was important to investigate the underlying mechanism of each gynecological tumor and whether the identified driver genes contributed to these diseases. Subsequently, a network was generated between the miRNAs and the identified driver genes, using the method of mining the Mir2 disease and Tarbase databases which provide information on miRNAs, diseases and the interactions between miRNAs and genes. Finally, the present study determined hub-genes and hub-miRNAs across the gynecological tumors to study the potential underlying mechanisms of the developments of gynecological tumors, which may shed light on different strategies for the design of biological targets for cancer therapies.

Materials and methods

Identification of gene expression datasets. In the present study, DEGs were identified between normal tissues and tumors extracted from the MERAV database from the National Center for Biotechnology Information GEO database (MERAV, http://merav.wi.mit.edu). The experimental samples for the present study are presented in Tables I and II. The following information was extracted from each identified study: GEO accession number, sample type, number of cases and controls, and gene expression data. Studies in which the microarray data were uncertain were excluded. The experimental protocol for the present study is presented in Fig. 1.

Integrated analysis of DEGs identified in the extracted databases. Information was extracted from the microarray datasets in MERAV which are presented in Tables I and II, respectively. Following the intersection of the microarray datasets, the DEGs were established between the normal and cancer tissues. In the present study, the degree of differential gene expression was measured by fold-change based on the Student's t-test. A fold-change value >2 or <0.5 and t-test $P<0.01$ for a gene was considered to be significant. The differential expression analysis was conducted using the Linear Models for Microarray Data package in R (20).

Protein interaction network. The DEGs were subsequently applied to the Human Protein Reference Database (21) (HPRD, www.hprd.org), to identify the more complex functional interactive driver genes of separate cancer types. Genes with interactions with each other were extracted from the DEGs as mentioned above (presented in Tables III-X). The PPI network is a useful research tool for investigating the cellular networks of protein interactions, and was downloaded from the HPRD. Cancer-associated gene-gene interaction networks were constructed by mapping the DEGs into the HPRD PPI network for each cancer (cervix tumor, ovarian tumor and endometrium tumor). To make it easier to identify the driver genes, the present study calculated the lines attached to each node, which was defined as the degree of the node. The nodes that exhibited degrees ≥4 were defined as driver genes. The nodes whose degree was ≥4 were considered to serve more complex roles in the development of the diseases of interest. These nodes were then extracted for the PPI network (Fig. 2). The present study constructed a connected network which contained the driver genes across the three cancer types. Through this method, it was determined whether the driver genes of the separate cancer types had any interaction with each other. The networks were constructed using Cytoscape version 3.3.0 (www.cytoscape.org).

miRNAs regulating gene network construction. The present study analyzed the association between miRNAs and the identified driver genes (Fig. 3). This process was performed by extracting a list of miRNAs which were associated with the type of cancer (cervical tumor, ovarian tumor or endometrial tumor) from the Mir2 Disease database (www.mir2disease.org) (22). Following this step, a network was created regarding the regulatory associations between the miRNA and the specific driver gene of each type of cancer in order to identify the hub-miRNAs of the gynecological tumors. The associations of the regulation were extracted from Tarbase (diana.cslab.ece.ntua.gr/tarbase) (23).

Functional and pathway enrichment analysis. In order to assess the functional relevance of the aforementioned DEGs, a pathway analysis was created based on the Database for Annotation, Visualization and Integrated Discovery (DAVID) (24). DAVID provides a useful tool to analyze large gene lists, including gene

Table I. Datasets from the Metabolic Gene Rapid Visualizer database (cervix).

Tissue type	Datasets
Normal, n=4	GSM176135, GSM175833, GSM176130, GSM176140
Tumor	
Squamous cell carcinoma, n=5	GSM152635, GSM277702, GSM46919, GSM102527, GSM152587
Squamous cell carcinoma non-keratinizing, n=5	GSM179907, GSM46942, GSM76614, GSM152580, GSM203742
Squamous cell carcinoma keratinizing, n=3	GSM117576, GSM152723, GSM152751
Adenoma, n=6	GSM179956,GSM152667,GSM152719,GSM179853,GSM325835,GSM203622

Table II. Datasets from the Metabolic Gene Rapid Visualizer database (ovary and endometrium).

	Datasets	
Tissue type	Ovary, n=4	Endometrium, n=22
Normal tissues	GSM175789 GSM176131 GSM176136 GSM176318	GSM175777, GSM175778, GSM175779, GSM175780, GSM175781, GSM175783, GSM175784,GSM175785, GSM176039, GSM176040, GSM176041, GSM176043, GSM176093, GSM176099, GSM176127, GSM176137, GSM176141, GSM176142, GSM176144, GSM176146, GSM176143, GSM176145,
Tissue type	Ovary serous adenocarcinoma, n=11	Endometrioid carcinoma, n=12
Tumors	GSM8897, GSM203626, GSM15267, GSM102445, GSM46831, GSM152577, GSM88973, GSM152581, GSM27769, GSM277737, GSM301703	GSM102425, GSM117582, GSM117586, GSM117590, GSM88952, GSM88966, GSM102469, GSM102492, GSM53058, GSM88978, GSM46923, GSM46937

ontology (GO) and pathway analysis. DEGs in different diseases were applied to this database in order to detect potentially represented functions. GO-categories were organized based on the GO database (25) (www. geneontology.org). In addition, pathway analysis was based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (26) (genome.jp/kegg). Significant categories were identified by expression analysis systematic explorer scores, a modified Fisher's exact P-value. The threshold for significance for a category was considered to be $P<0.01$, with >4 genes for the corresponding term.

Survival analysis. The present study used TCGA database to extract clinical information and gene expression profile information. At the start of the analysis, the expression values of each driver gene were listed, which were identified via the PPI network. To find the median level of gene expression, the samples were divided into two groups by median of expression (high expression group and low expression group). Additionally, the corresponding clinical information of each sample was extracted. Survival data representing time between initial diagnosis and mortality were downloaded directly from TCGA data portal (tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp) (27). With this information, the present study was able to estimate the association between the identified driver genes of the three types of cancer mentioned above and the survival rates of patients. All analyses were conducted using custom-written code in R (www.r-project.org).

Results

Integrated analysis of multiple studies to establish the driver genes in cancer. There are multiple genes that contribute to

Table III. Driver genes identified by integrated analysis of the microarray datasets (cervical squamous cell carcinoma).

Gene					
RB1	HTRA1	MTOR	CLDN5	NARF	PURA
MCM7	KPNA2	PLSCR4	CYBA	NCAPD2	RBM8A
MCM2	LMNB1	PRKD1	DCUN1D1	NCF4	RECK
PLK1	MEIS1	PSMA5	DDAH2	NME4	REV3L
AR	NCOA1	PSMB10	DMPK	NPLOC4	RFC3
PPP1CA	PBX1	PSMB9	EPS8	NR2F1	RNF126
ABL1	PIAS3	PSMD2	EXOSC5	NR2F2	RPA3
LMNA	POLA2	RACGAP1	GABBR1	NRAS	RRM1
PTN	PPP1R14A	RTN3	GAS6	NTF3	RRM2
TRIP13	AXL	SNRPB	GCH1	NTRK2	SAT2
CAV1	BUB1B	TOR1AIP1	GCHFR	NUB1	SDC2
CDC20	CCL14	TUBA4A	GLRX3	NUP210	SEC24A
CDC6	CCR5	UBTF	GMFB	NUP50	SELENBP1
FLNA	COL4A5	USP6NL	GOLGA2	PAFAH1B3	SERBP1
FXR2	CSNK1D	UTP3	HOXD13	PAK2	SH3BP5
ZHX1	DBF4	ACTN4	ILK	PAM	SMC4
CCNA2	DVL3	ADAM10	KANK1	PCGF2	SNRPD1
DGKZ	EFEMP2	ANTXR2	LAPTM5	PHACTR4	SNTB2
MCM10	EIF4EBP1	ARHGAP17	LDB2	PLK2	SNX27
MCM6	EZH2	ASPM	LDOC1	PNO1	SPIN1
PCNA	FAM46A	BID	LMO4	PNP	SSSCA1
RBPMS	HOXD10	BMP4	LRP1	PPIA	STXBP2
RPS6KA1	HSPA4	BNIP2	LRP6	PPIH	SUB1
SAT1	ITGB3BP	C1QA	LRRC41	PRPF18	TALDO1
BUB1	KLF6	CBX4	LZTS2	PSMA6	TGFBR3
CSNK1E	MAD2L2	CCNE1	MAGEH1	PSMB7	TNFRSF1A
DCN	MAP2K4	CCR1	MELK	PSMD4	UFD1L
FGFR1	MAPK10	CDC42BPA	MPDZ	PSME3	WSB2
FXYD1	MCM5	CENPE	MTA1	PSMF1	XPNPEP1
GMNN	MITF	CHFR	MYCBP	PSTPIP1	YLPM1
HOXA10	MMP9	CIB1	MYL9	PTTG1	ZMIZ1

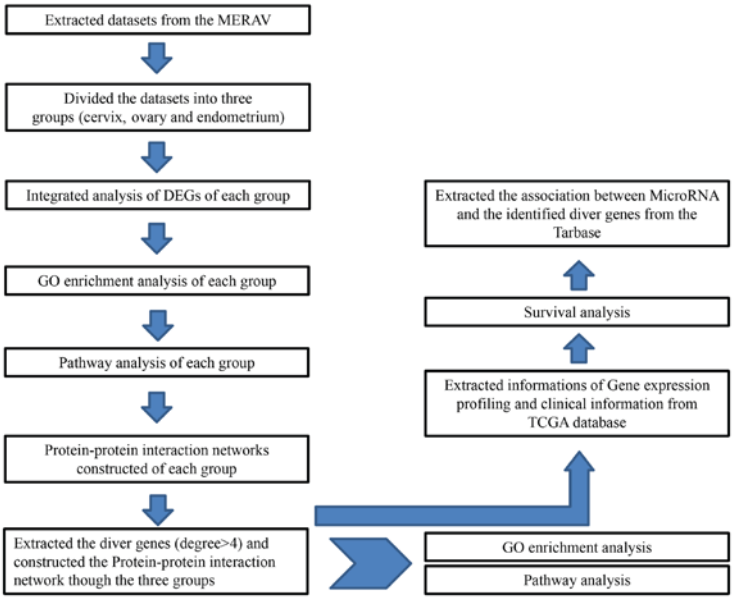

Figure 1. Experimental protocol of the present study. DEG, differentially express genes; GO, gene ontology; MERAV, Metabolic Gene Rapid Visualizer database; TGCA, The Cancer Genome Atlas.

Table IV. Driver genes identified by integrated analysis of the microarray datasets (cervical keratinized squamous cell carcinoma).

Gene					
FYN	ADAM10	ARHGAP17	HSPB2	PHF1	TMOD1
ZHX1	ADAM17	ARMCX2	ID4	PIK3C2B	TMSB10
ABL1	ANXA6	BIN1	LDB2	PIP5K1C	TPD52
BCL2L1	AXL	CBX3	LDOC1	PNP	UBTF
FXR2	BCL11A	CLDN5	LMO4	PSME3	ZHX2
TBP	CSNK1E	CNN3	LRP1	PSMF1	ZMIZ1
AR	DMPK	CNNM3	LRP6	PTOV1	ZNF76
BARD1	ITGB3BP	CNTNAP1	LSM5	PTPN12	
BID	KPNA6	CRYAB	MAGI2	RAE1	
DDX24	MAD2L2	CSE1L	MAPK10	REV3L	
NCOA1	MCL1	CSTF1	MIS12	RUNX1T1	
PDGFRB	NR2F1	EFEMP2	MPDZ	SDC2	
PRKD1	NTRK2	EXOSC5	MTA1	SFRP1	
PSEN1	PPP1R14A	FGFR1	MYCBP	SH3BP5	
RBPMS	PTN	FXYD1	NPDC1	TAF9	
SPTAN1	RTN3	FZD6	NR2F2	TCF7L2	
TCF4	SYK	GAS6	NTF3	TERF1	
TGFA	VIM	GDI1	NUDT21	TFDP1	
A2M	ANTXR2	GTF3C3	PBX1	TGFBR3	
ACP1	AQP1	HOXA10	PDGFD	TLN2	

Table V. Driver genes identified by integrated analysis of the microarray datasets (cervical adenocarcinoma G3).

Gene					
AR	BAD	PLD2	CIB1	MAPK10	SERPINA1
CAV1	BAHD1	PPA1	CLDN5	MED14	SF1
FLNA	C1QBP	PRKD1	CUL4B	MPDZ	SMO
PPP1CA	CPSF6	SAT1	DMPK	MYL9	SPINT2
NCK2	CSNK1D	SMAD1	EFNB1	NR2F1	SSBP3
PLSCR1	DOCK1	SNAP23	F3	NTF3	SSR1
SUMO4	DVL2	TAF1D	GDI1	PCGF2	STAM
LMNA	FXR2	TAF9	HOXA10	PDPK1	SYNE1
LRP1	FXYD1	TCF4	HOXD10	PHACTR4	TCF7L2
PSEN1	ILK	WIPI1	HOXD13	PHYHIP	TGFBR3
PTN	LDB1	ACVR2A	HSPA1B	PLSCR4	TMF1
CSNK1E	LMO4	ANTXR2	HSPBAP1	PNPLA2	UBTF
DVL3	MAP2K4	ATG12	KANK1	PPP1R10	VAMP8
MMP14	NCOA1	CD82	KPNA6	PTCH2	WASF1
PPP1R14A	NTRK2	CDC42BPA	LDB2	RNF138	WASF2
ALDOA	PBX1	CDC42EP1	LRP6	RUNX1T1	ZHX1

the cause of the aforementioned cancer types and, therefore, no single gene is a determining factor in diagnosis. It was identified that each type of cancer was driven by different variations of genes that serve key roles during the development of pathology. However, no single gene may explain the heterogeneity of each type of cancer. In the case of cervical cancer, 186 genes in squamous cell carcinoma of the cervix (Table III), 107 genes in keratinized squamous cell carcinoma of the cervix (Table IV), 96 genes in cervical adenocarcinoma Grade 3 (Table V), 133 genes in non-keratinized squamous cell carcinoma of the cervix (Table VI) and 203 genes in cervical adenocarcinoma Grade 2 (Table VII) were identified to be important. In addition, 120 genes and 76 genes were established, respectively, in adenocarcinoma of the ovary Grade 2

Table VI. Driver genes identified by integrated analysis of the microarray datasets (cervical non-keratinized squamous cell carcinoma).

Gene					
AR	FXR2	FOXO1	TLR2	FBN2	NTF3
ABL1	ILK	GMNN	TXNDC9	FGR	NTRK2
CAV1	LMNA	HOXD10	XRCC4	FXYD1	NUBP1
CHD3	MEIS1	ICAM3	YAP1	GDI1	PALLD
HIF1A	NCOA1	ITGB2	ADCY6	HCLS1	PDPK1
PTPN6	PAG1	LCP2	ADI1	HLA-DMB	PGK1
SAT1	PBX1	LRP1	AGTPBP1	HLA-DRA	PGLS
FLNA	PIAS1	MAFG	ANTXR2	HOXD13	PIK3R3
HOXA10	PSEN1	MPDZ	ANXA6	HSPB2	PLTP
PLSCR1	PTN	NDN	ARHGDIB	LCP1	PNP
RAF1	WASF2	NR2F2	CDC37	LDOC1	PRRX1
DCN	ZHX1	PAICS	CITED2	LILRB2	RAB11FIP2
EZR	ACTR3	PLSCR4	CLDN5	LRP6	RAB18
MMP14	BIN1	PPP1R14A	CNN3	MAPK10	RFXANK
PDGFRB	C1QB	PPP2R1A	COL4A5	MED14	RUNX1T1
ABCA1	C1QC	PRDX2	DOCK1	MTA1	SAT2
C1QA	CSNK1D	SNTB2	DVL2	MYO5B	SEPHS1
CSNK1E	DGKZ	SSSCA1	ENO1	NARF	SF1
DMPK	DVL3	TCF4	FAM46A	NISCH	
ELN	EFEMP2	TLR1	FBLN1	TICAM1	
SNX2	SYNE1	TCF7L2	VTA1	TRAP1	
TMEM8B	TMOD1	TMSB10	TPD52	SH3BP5	
WASF3	ZNF76	TEAD3	TIMP2	NR2F1	

and Grade 3 (Tables VIII and IX). A total of 168 genes were established in endometrial carcinoma (Table X).

Integrated PPI (protein-protein interactions) network construction. Based on the HPRD, the interaction network of the identified driver genes was constructed, which consisted of 101 nodes (genes that form associations) and 185 edges (biological association) (Fig. 2). Genes with a higher degree of association (degree ≥4) were observed to be larger in size, and included the genes CDK1, CAV1, ZBTB16, Jun proto-oncogene AP-1 transcription factor subunit (JUN), RAF1, RB1, mini-chromosome maintenance complex component 2 (MCM2), AR, ABL1, LMNA, FLNA, DCN, FYN, SMAD1, LRP1, PSEN1, EP300, CTNNB1, collagen type I α1 chain (COL1A1) and FOS. Through this method, it was identified that driver genes in each gynecological cancer have contact interactions.

Comprehensive analysis of miRNA regulation and the selected driver genes. Fig. 3 illustrates that certain miRNAs serve important roles in regulating the driver genes. In the present study, it was demonstrated that a number of miRNAs regulate separate networks [for example the let7 family, miRNA (miR)-23b, miR-21, miR-214 and miR-218]. miRNAs that were confirmed to be significant in cervical cancer, including let7c and let7b, are also found to be associated with the other two cancers in this study. This information may be important in establishing the connections between the three gynecological cancer types, which may be used in the development of targets for further research and diagnosis.

Functional and pathway enrichment analysis. GO analysis revealed that the identified genes of cervical tumors, ovarian tumors and endometrial tumors were predominantly involved in the illustrated biological processes (Fig. 4). The top three significant biological processes of cervical cancer were 'mitotic cell cycle', 'cell cycle' and 'cell cycle process', while for ovarian cancer, the biological processes consisted of 'cell cycle process', 'cell cycle phase' and 'macromolecule metabolic process'. For the progression of endometrial cancer, the top three biological processes observed to be at fault for cancer progression were 'response to organic substance', 'regulation of cell proliferation' and 'skeletal system development'.

Using the method of pathway analysis, it was revealed that genes in cervical cancer were significantly enriched in 'cell cycle', 'pathways in cancer' and 'DNA replication'. Ovarian cancer was observed to be significantly enriched in 'MAPK signaling pathway', 'cell cycle' and 'oocyte maturation'. Endometrial cancer was observed to be significantly enriched in 'pathways in cancer', 'focal adhesion' and 'complement and coagulation cascades' (Fig. 5).

Survival analysis of patients with gynecological tumor. Fig. 6 illustrates the association between survival time and survival rate in the high and low expression groups. The genes MCM2,

Table VII. Driver genes identified by integrated analysis of the microarray datasets (cervical adenocarcinoma G2).

Gene					
ABL1	HSPA5	ASAP1	PSMF1	ASS1	EHD2
AR	HTRA1	AXL	QKI	ATRX	ENAH
CAV1	LMNA	BCR	RAB4A	AURKA	ENO1
PPP1CA	MEIS1	BGN	RNF138	AURKB	ERBB3
FLNA	NTRK2	BMP4	SDC2	BIN1	FBLN1
FYN	PRNP	BRCA2	SMARCE1	BIRC5	GAS6
MMP2	PTPN12	CDKN2A	SNAP29	CAPZB	GLRX3
SMAD1	SMAD5	CSNK1E	TAF7	CAV2	GOLGA2
NCK2	TAF9	DMPK	TCF4	CBX4	GTF2I
RB1	TTF2	DOCK1	TGFBR3	CD81	HAT1
PTN	DVL2	DR1	THBS2	CDT1	HOXD10
PTPN6	EFEMP2	FGFR1	TIFA	CEP76	HSPA1B
SMAD7	FXR2	FXYD1	TIMP2	CLDN5	HSPB2
SUMO4	HOXA10	GDF5	TNFRSF1A	CLU	IDE
A2M	HOXD13	GNA12	ZHX1	CNN3	IFI35
AP1M1	LRP1	KIDINS220	ADI1	CNTNAP1	IFNAR1
CDC5L	NCOA1	LDOC1	AHNAK	COL4A5	ILK
EZR	NOTCH2	LRP6	ALDOA	COL6A3	IQGAP1
MMP14	PBX1	MAFG	ANTXR1	COX5A	JAG1
PIAS1	PDGFRB	MAP2K4	ANTXR2	CUL4B	KANK1
CD2AP	PRKD1	MAPK10	ANXA6	CXCL12	KDM2A
CDH1	SAT1	MEF2C	AQP1	DCLRE1A	KPNA6
DCN	WASF2	POLE3	ARHGAP17	DDX24	LCAT
DRAP1	YAP1	PPP1R14A	ARHGEF6	EFNB1	MAD2L1BP
ELN	ACVR2A	PRRX1	ASH1L	EFS	MAP3K3
MCM4	NR2F2	PLSCR4	RUNX1T1	SYNE1	WNK1
MED14	NTF3	PPA1	SALL2	TEAD3	YLPM1
MPDZ	NUDT21	PPP1R10	SAT2	TERF1	ZMIZ1
MSN	PALB2	PPP2R1A	SETD7	THBS3	
MYCBP2	PALLD	PSMB10	SH3BP5	TMEM8B	
MYO5B	PBX3	PURA	SH3KBP1	TSPAN4	
NFE2L1	PDGFD	RAB11FIP1	SKAP1	TWIST2	
NMI	PHACTR4	RAB11FIP2	SPARCL1	UBTF	
NPHS2	PIP4K2B	RBPJ	STX3	VGLL4	
NR2F1	PKD2	REPS2	STX7	WFDC2	

MMP2, COL1A1 and JUN are presented in the figure, and it was observed that the driver genes of the expression groups were able to divide each of the target cancer types into two groups, one of which contained the high expression group with the other containing the low expression group. Therefore, in order to determine whether the driver genes had a key role in the development of gynecological tumors and the connective function of separate cancer types, the present study aimed to identify the association between the target cancer driver genes and other types of gynecological cancer.

Discussion

The principal challenge of high-throughput cancer genomics is to identify specific driver genes and the underlying mechanisms of carcinogenesis, apart from the vast quantity of heterogeneous genomic alteration data. Numerous studies have focused on identifying individual functional modules or pathways involved in cancer (28-30). Based on this methodology, the analysis of the present study focused specifically on DEGs in order to reveal the transcriptional responses of gynecological tumors. The results of this analysis suggested that the common biological processes of cancer of the cervix, ovary and endometrium were those involved in the cell cycle and the regulation of macromolecule metabolism.

The cell cycle is the progression of biochemical and morphological phases and events that occur in a cell during successive cell replication or nuclear replication. Research has shown that interference with cell cycle components may

Table VIII. Driver genes identified by integrated analysis of the microarray datasets (adenocarcinoma of the ovary Grade 2).

Gene					
JUN	MEF2C	HSPA1A	CNNM3	GNE	PHF1
FXR2	NCOA2	HTRA1	COX5A	GNG4	PKD2
RAF1	NIF3L1	IKZF4	CRY2	GPRASP1	PLA2G16
RBPMS	PCBD1	LIFR	CTF1	HMGA1	PLK1
ZBTB16	PDGFRA	MAPK10	CTSD	HSPA2	PTPN13
PRKACA	PRTFDC1	MYO15A	DCN	ICAM3	RBBP8
CAV1	STAT5A	NFE2L1	DST	IGFBP4	RBP1
MAP3K3	APBB1	NR2F6	ELF3	IRS1	SDC2
MAP3K5	C1R	PER1	ELK1	KIAA1217	SGK1
NCOA1	C1S	PTPN6	ENAH	MAFG	SH3BP5
PDGFRB	CALCOCO2	SERPING1	ENG	MRAS	SMC3
SIN3A	CD2AP	SIN3B	EPS8	NBL1	SNCA
ABLIM1	DCTN1	TGFBR3	ETV6	NFATC4	SNRNP70
DDX17	DMPK	TSC22D3	EYA2	NINL	SPOP
FEZ1	DVL2	UBQLN1	FLAD1	NR2F2	SPTBN1
GATA4	FHL2	ACTA2	FOXO1	OLFML3	SPTBN2
GOLGA2	FLNA	BEGAIN	FOXO3	PAICS	ST13
LRP1	FXYD1	CCT5	FTH1	PDGFD	STRBP
TCF4	THRA	TPM2	TXN	USP13	ZC3H10
TEAD1	TOP2A	TRIM21	TXNDC9	WTIP	ZFPM2

Table IX. Driver genes identified by integrated analysis of the microarray datasets (adenocarcinoma of the ovary Grade 3).

Gene					
CDK1	HLA-DRA	CD14	FCGR2B	PDGFD	NR2F2
AURKB	ICAM3	CDC20	FOS	SLPI	
CAV1	KRT7	CDH1	GCA	SMC4	
PTPN6	MAD2L1	CDKN2A	GNE	SOX9	
ZBTB16	MAL2	CEBPG	GPRASP1	SPINT1	
BCL2L1	MAP3K5	CENPA	HLA-DMB	ST14	
HSPA1A	PDGFRA	CKS2	HLA-DRB1	STRBP	
IRS1	PDGFRB	CLDN1	LAPTM5	TACC1	
ITGB2	PMAIP1	CLDN3	LCP1	TOP2A	
MCM2	RACGAP1	CRIP1	LRP1	TRIP13	
NDC80	RBPMS	CTSS	MSLN	TYROBP	
SYK	TPD52	CXCR4	MUC1	ZWINT	
TPD52L1	ALOX5	DBF4	MUC16	ECT2	
BCL11A	ALOX5AP	DSC2	NCAPD2	CCNB1	
CCNB2	BIK	DSG2	NR2F1	ERBB3	

lead to tumor formation (31). Certain cell cycle inhibitors, including retinoblastoma protein and tumor protein 53 may mutate during replication, causing the cell to proliferate uncontrollably, ultimately resulting in a tumor. Furthermore, the proportion of active cell division in tumors is much higher compared with the rate in normal tissue.

To clarify the hub genes in ovarian cancer, cervical cancer and endometrial cancer, DEGs were predicted to be biomarkers for each cancer using PPI networks. It is considered that hub nodes are genes that are highly connected with other genes and have been predicted to serve key roles in numerous networks. In addition, highly connected hub genes were proposed to have a considerable role in biological development. Hub nodes have more complex interactions compared with those of other nodes, which indicates that they have pivotal roles in the underlying mechanisms of disease. In addition, certain identified biomarkers of each type of cancer were extracted from each network and these driver genes were placed into one PPI

Table X. Driver genes identified by the integrated analysis of the microarray datasets (endometrial carcinoma).

Gene					
EP300	CDKN2A	F2R	AMFR	EPN3	MMP11
JUN	COL3A1	FZD5	AXL	EPR1	MMP26
CAV1	EGR1	HLA-DMB	BCL11A	FOSB	MYO5B
CTNNB1	ERBB4	HOXA10	BCL2A1	GALNT10	NRG2
ABL1	FBLN1	ID1	BIK	GAS6	NRXN2
AR	FBN1	ID4	BLNK	GATA2	PCOLCE
TCF4	FLNA	IDE	C1R	GCH1	PDGFRB
THBS1	FOXO1	INADL	C1S	GCHFR	PKD2
TUBA4A	HLA-DRA	JUND	C3AR1	GPI	PNP
ATXN1	ID3	LMO4	CCND2	GPRASP1	PPP1R14A
COL1A1	IGFBP5	LNX1	CDH11	HLA-DQB1	PRDM1
DCN	LAMB3	NCALD	CDKN1A	HLA-DRB1	PSTPIP2
LRP1	MITF	NCF2	CDKN2C	HLF	PTGDS
C3	MYC	NR2F2	CFB	HOXA9	PTGS2
COL7A1	PLAT	PDGFRA	CGN	ID2	R3HDM2
FBLN2	RUNX1T1	PLEKHF2	CLEC3B	IGFBP4	RAB25
FOS	S100A8	PTPN13	CLK1	IGFBP6	RAB3IP
GNAI2	SERPINA1	RAB8B	CXADR	IL33	RAPGEF6
IGF1	SYK	RABAC1	CXCL10	IRS1	S100A9
LAMC2	TGFB1I1	ROR2	DNM1	KLF5	SCRIB
MUC1	CD14	SFN	DPYSL2	LAPTM5	SEC24D
NID1	COL5A1	SFRP1	ECM1	LDB2	SNTB2
PRKD1	CRMP1	TFAP2A	EDNRA	LUC7L3	SOX9
PTPN12	DBP	TJP2	EFEMP2	MAFB	SPINT1
VCAN	DDR2	TRPC1	EFS	MAL2	SPP1
CD74	F10	WNT5A	ENO2	MAPK10	ST14
SYTL1	TJP3	TLR3	TRO	WASF2	WNT4
TBL1X	TLR2	TPD52	USP54	WNT2	ZEB1

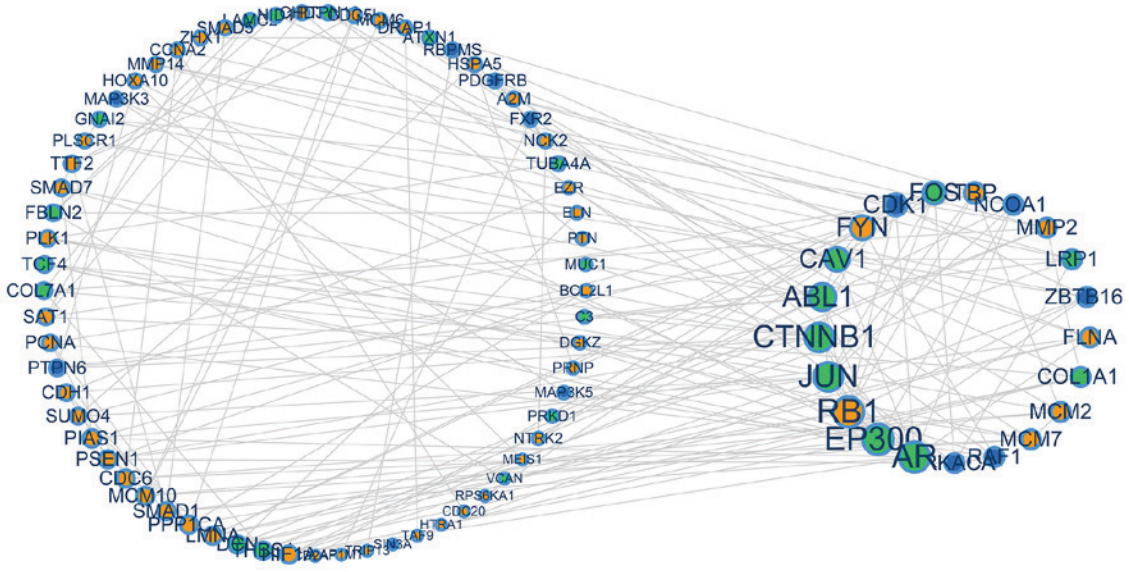

Figure 2. Protein-protein interaction networks of the DEGs identified by integrated analysis of the microarray databases throughout cancer of the cervix, ovary or endometrium. Each cancer holds a number of DEGs. Driver genes were extracted from the DEGs, whose degree (the number of lines attached to each node) was ≥4. The orange dots represent cervical carcinoma, green dots represent ovarian carcinoma and blue dots represent endometrial carcinoma. Genes with a higher degree of association exhibit a larger node size. Each biological association (an edge) between two genes (nodes) was supported by at least one reference from the literature or information stored in the Human Protein Reference Database. DEGS, differentially expressed genes.

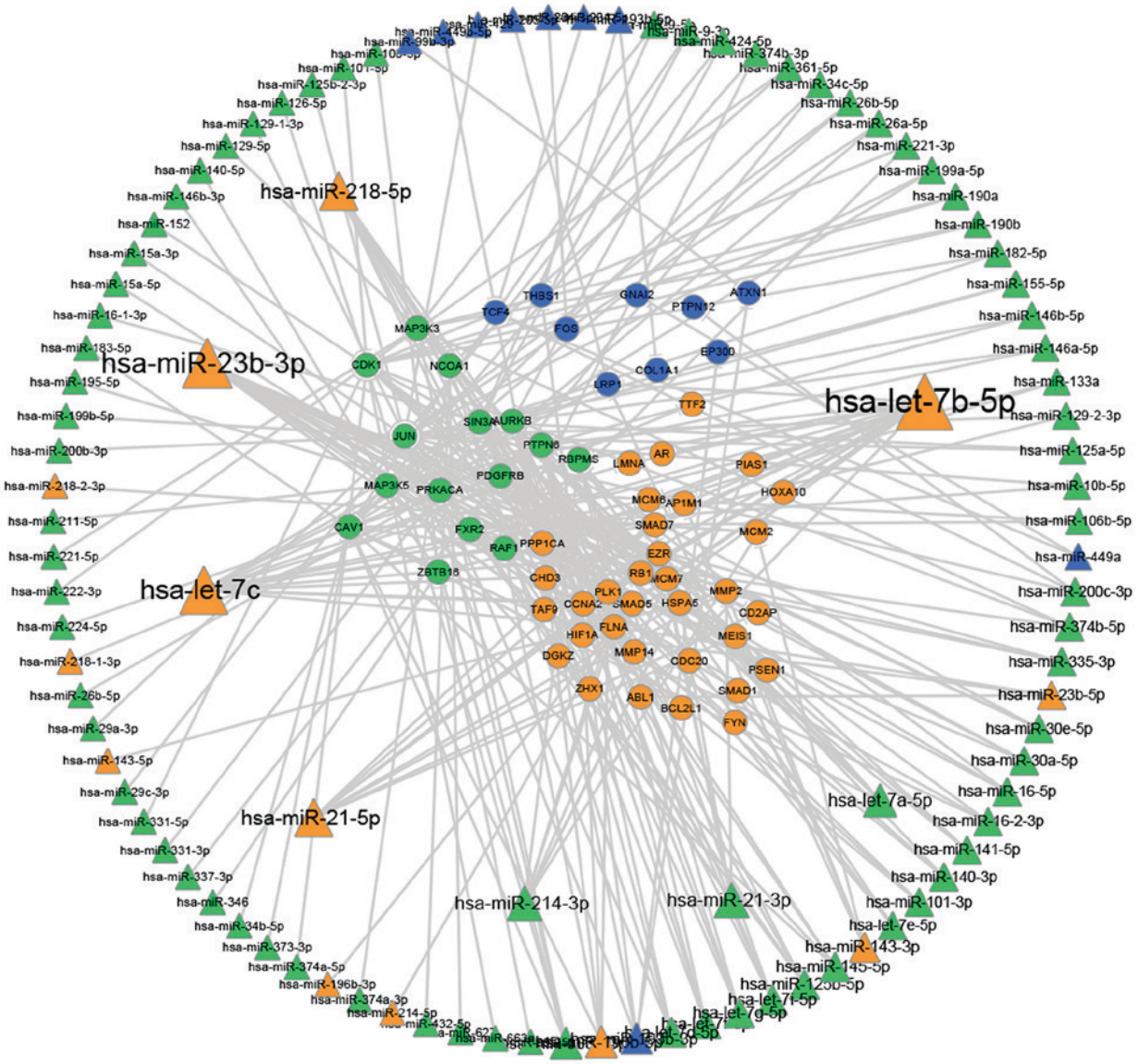

Figure 3. Network construction of miRNAs to driver genes. The miRNA dataset was downloaded from the Mir2 Disease database (www.mir2disease.org). The miRNAs presented in the figure are associated with cancer of the cervix, ovary or endometrium. Triangular nodes represent miRNAs. Circular nodes represent genes. Orange dots represent cervical carcinoma, green dots represent ovarian carcinoma and blue dots represent endometrial carcinoma. The degree for each dot is represented by the size of the node. miRNA/miR, microRNA.

network with the duplication hub genes eliminated. Therefore, the particular hub genes of each gynecological cancer and the connection nodes across the three types of cancers may be identified. Accordingly, the identification of hub genes and hub connected genes involved in each gynecological cancer may lead to the discovery of the association across ovarian cancer, cervical cancer and endometrial cancer, and may lead to the development of effective diagnostic and therapeutic approaches.

In order to ascertain a causal association across the three types of gynecological cancer, the present study extracted clinical information and gene expression profile information from TCGA database, and used the hub connected genes identified in the PPI network to perform survival analysis. In the present study, four noteworthy genes were identified, including MCM2, MMP2, COL1A1 and JUN.

The present study demonstrated that MCM2 may serve a key role in cervical cancer. A poor prognosis was associated with lower expression. Furthermore, MCM2 was highly connected with ovarian cancer and endometrial cancer. The results suggested that MCM2 is a component of the DNA replication licensing complex, with a rich binding surface that directs multiple regulatory interactions of cancer significance, marking DNA replication origins during the G1 phase of the cell cycle for use in the subsequent S-phase. A deficiency of MCM2 results in death or morbidity in the absence of an overt tumor (32). These processes of DNA replication have been studied and used as therapeutic targets. Simon and Schwacha (33) suggested that MCM2 was a promising target for blocking the proliferation of cancerous and precancerous cells.

In the present study, MMP2 was identified to be essential in causing cervical cancer. MMPs are zinc-containing endopeptidases with an extensive range of substrate specificities. These enzymes are able to degrade various components of extracellular matrix (ECM) proteins. In photocarcinogenesis,

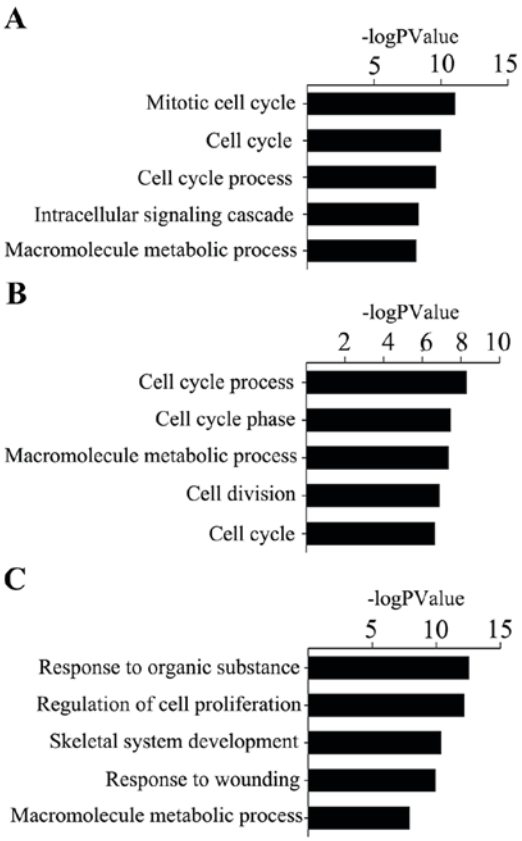

Figure 4. (A) GO terms of cervical cancer driver genes. (B) GO terms of ovarian cancer driver genes. (C) GO terms of endometrial carcinoma driver genes. GO, gene ontology.

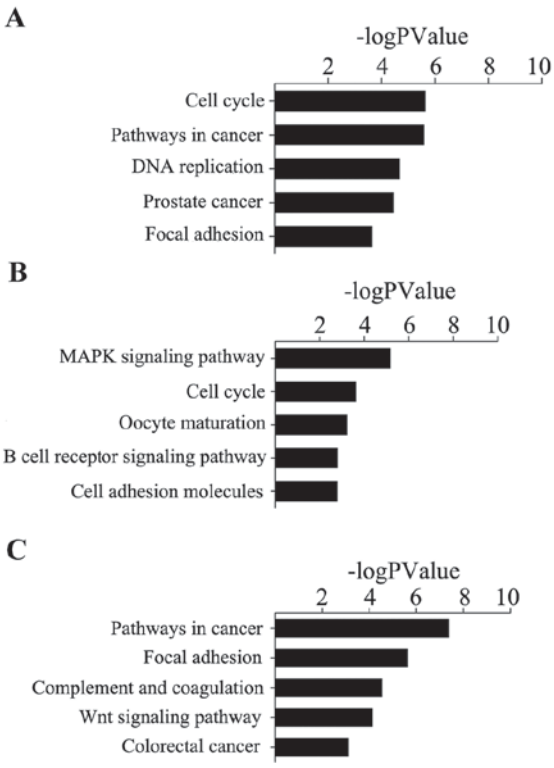

Figure 5. (A) KEGG pathway functional annotation of cervical cancer driver genes. (B) KEGG pathway functional annotation of ovarian cancer driver genes. (C) KEGG pathway functional annotation of endometrial carcinoma driver genes. KEGG, Kyoto Encyclopedia of Genes and Genomes.

degradation of the ECM is the initial step towards tumor cell invasion, to intrude in the basement membrane and the surrounding stroma that primarily comprises fibrillary collagens. Additionally, MMP2 is involved in angiogenesis, which promotes cancer cell growth and migration (34).

COL1A1 and COL1A2 encode the α1 and α2 chains of type I collagen, respectively (35). The primary constituents of the ECM are collagens, adhesive glycoproteins and proteoglycans (36). Specific interactions between cells and ECM-mediated cell-surface-associated components and transmembrane molecules result in the control of cellular activities, including adhesion and migration (37). Collagen is the primary component of the ECM, which serves pivotal roles in maintaining skin and vessel elasticity, and increasing cartilage lubricity (38). Upregulation of type II collagen expression may contribute to ovarian cancer metastasis and biological processes, including cell proliferation, invasion and migration (39). The oncogene JUN is the putative transforming gene of avian sarcoma virus 17, which is the most extensively studied protein of the activator protein-1 complex and is involved in numerous cell activities, including proliferation, apoptosis, survival, tumorigenesis and tissue morphogenesis. The present study identified that COL1A1 was important in ovarian cancer, which was highly connected with cervical and endometrial cancer. Therefore, COL1A1 and JUN may be potentially important associated genes of the three types of gynecological malignancies.

miRNAs are small noncoding regulatory RNAs that downregulate transcription by targeting specific mRNAs. Furthermore, the present study identified that certain miRNAs were highly associated with hub connected genes, including let7, which is one of the founding members of the miRNA family. This miRNA was first identified in *Caenorhabditis elegans*. Lee and Dutta (40) identified six functional let7 target sites in the 3'-untranslated region of high mobility group AT-hook 2 (HMGA2), which reduced HMGA2 expression and cell proliferation in a lung cancer cell line. Using genome-wide mRNA expression analysis, Mi *et al* (41) identified that miRNA let7B was downregulated in acute lymphoblastic leukemia (ALL) compared with acute myeloid leukemia (AML). Quantitative polymerase chain reaction analysis confirmed the downregulation of let7B in ALL samples compared with AML samples and normal controls.

The present study identified that let7a, let7b and let7c had strong connections with the hub genes and that these miRNAs may serve an important part of the potential mechanism, which may explain the connections across the hub genes.

Overall, the present study identified a number of DEGs associated with gynecological cancer, in addition to the functions and signaling pathways in which these genes were involved. Comprehensive network analyses of the dysregulated gene expression in gynecological cancers identified a series of hub genes and the connection genes across ovarian cancer, cervical cancer and endometrial cancer in a PPI network. Subsequently, this study confirmed the driver genes by survival analysis using the TCGA database. Comprehensive network analyses

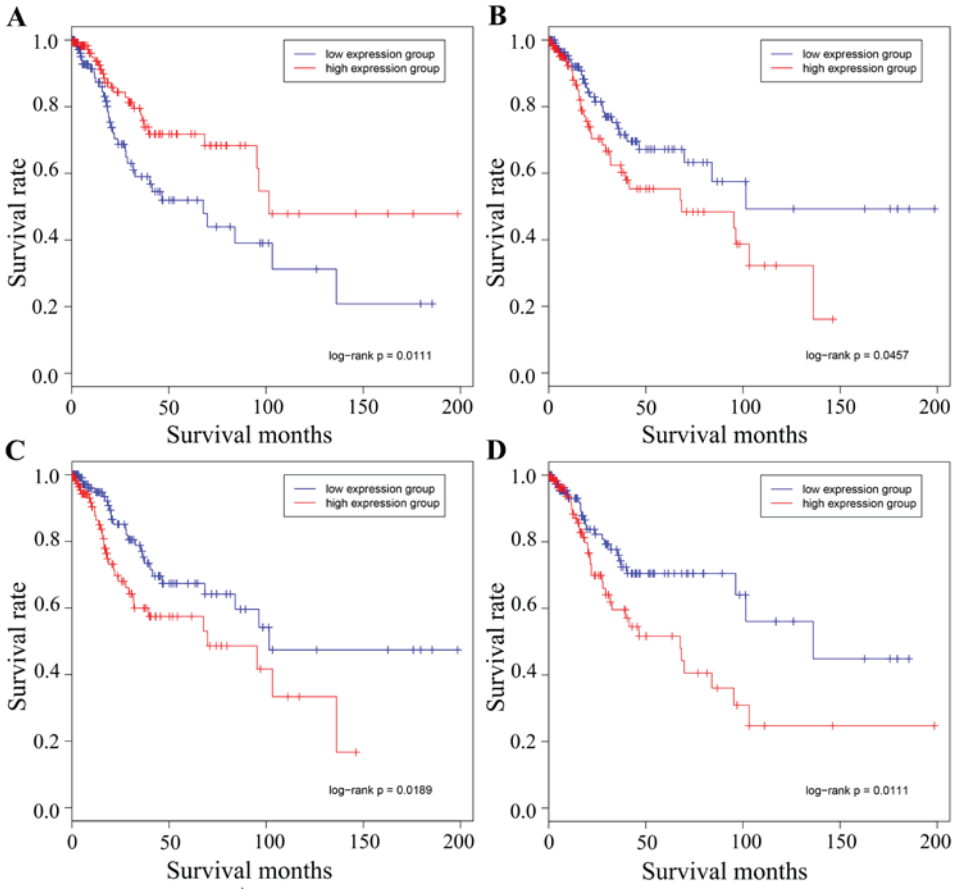

Figure 6. Survival analysis of the different cancer types using the representative driver genes. Survival data representing time between initial diagnosis and mortality were downloaded directly from TCGA data portal. The red line represents the high expression group and the blue line represents the low expression group. (A) Cervical hub-gene MCM2 in cervical cancer. high and low expression of MCM2 divided the samples into two groups, with 133 and 144 samples in each group, respectively. (B) Cervical hub-gene MMP2 in cervical cancer, whose high and low expression divided the group into two, with 142 and 142 samples in each group, respectively. (C) Ovarian hub-gene COL1A1 in cervical cancer, whose high and low expression divided the group into two, with 143 and 141 samples in each group, respectively. (D) Ovarian hub-gene JUN in cervical cancer, whose high and low expression divided the group into two, with 141 and 144 samples in each group, respectively. MCM2, minichromosome maintenance complex component 2; MMP2, matrix metalloproteinase 2; COL1A1, collagen type I α1 chain; TCGA, The Cancer Genome Atlas.

of miRNAs and connection driver genes identified certain miRNAs which may be potential therapeutic and prevention targets of gynecological cancer. In addition, the present study demonstrated the associations across the different gynecological cancers, which may be useful for identifying potential useful diagnostic markers and novel therapeutic targets. The results of this study may provide an insight into the underlying mechanism of the aforementioned gynecological cancers and may lead to further improvement in diagnosis and treatment of them.

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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

MY and JW conceived and designed the study; MY, LL and JL performed the experiments and analyzed the data. MY wrote the paper, and JW revised the manuscript and gave final approval of the version to be published.

Ethics approval and consent to participate

The present study was approved by the Clinical Research Ethics Committee of the Affiliated Zhuzhou Hospital Xiangya Medical College CSU (Zhuzhou, China), and written informed consent was obtained from all participants.

Consent for publication

Written informed consent was obtained from all volunteers for the publication of any associated data.

Competing interests

The authors declare that they have no competing interests.

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