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Exploring prognostic genes in ovarian cancer stage-related coexpression network modules

Lili Yang, MD[a], Jili Jing, MD[b], Liqun Sun, MD[c], Ying Yue, MD[d,*]

Abstract

Identification of meaningful cluster modules of differential genes or representative biomarkers related to the stages of ovarian cancer (OC) is pivotal, which may help to detect mechanisms of OC progression and evaluate OC patients' prognosis.

We downloaded gene expression data and the corresponding clinical information of OC patients from The Cancer Genome Atlas (TCGA) database, which included 379 ovarian cancer patients. Differentially expressed genes (DEGs) of OC patients between stages were picked out using R. There were 731 differential genes between ovarian cancer stage II and stage III (DEGs $_{II-III}$) and 563 differential genes between ovarian cancer stage III and stage IV (DEGs $_{III-IV}$), then we performed GO analysis and Kyoto Encyclopedia of Gene and Genome (KEGG) pathway analysis using Database for Annotation, Visualization and Integrated Discovery (DAVID). Moreover, CytoHubba was used to detect the top 20 hub genes in DEGs $_{II-III}$ and DEGs $_{III-IV}$, followed Cytoscape with search tool for the retrieval of interacting genes (STRING) and MCODE plug-in was utilized to construct protein-protein interaction (PPI) modules of these genes. Three important coexpression modules of DEGs $_{II-III}$ and 3 more meaningful modules of DEGs $_{III-IV}$ were detected from PPI network using molecular complex detection (MCODE) tool. In addition, 5 hub genes in these stage-related DEGs modules with worse overall survival were selected, including *COL3A1, COL1A1, COL1A2, KRAS*, *NRAS*. This bioinformatics analysis demonstrated that stage-related prognostic DEGs, such as *COL3A1, COL1A1, COL1A2, KRAS,* and *NRAS* might play an unfavorable role in the development as well as metastasis of ovarian cancer. Furthermore, they need to be experimentally verified as a new biomarker to predict OC patient prognosis.

Abbreviations: CA125 = cancer antigen 125, DAVID = Database for Annotation, Visualization and Integrated Discovery, DEGs = differentially expressed genes, DEGs $_{II-III}$ = differential genes between ovarian cancer stage II and stage III, DEGs $_{III-IV}$ = differential genes between ovarian cancer stage III and stage IV, FIGO = International Federation of Gynecology and Obstetrics, HGS-OvCa = high grade serous ovarian cystadenocarcinoma, KEGG = Kyoto Encyclopedia of Gene and Genome pathway, MCODE = molecular complex detection, OC = ovarian cancer, PPI = protein-protein interaction, STRING = search tool for the retrieval of interacting genes.

Keywords: differential genes, ovarian cancer, overall survival, prognosis

1. Introduction

Ovarian cancer (OC) is the most lethal gynecological cancer and the fifth most common cause of cancer-related death among women in the United States.[1] Due to latent symptoms and lack of reliable early screening means, most OC patients are diagnosed at an advanced stage (stage III–IV; International Federation of Gynecology and Obstetrics, FIGO).[2] Early detection and diagnosis of OC remain the main target for successful treatment. For advanced-stage OC are much more likely to have a poor prognosis, exploring gene expression characteristics related to OC stage is critical.

Currently, the only biomarker that is widely used in clinical practice is cancer antigen 125 (CA125).[3] This high MW glycoprotein CA125 is elevated in 90% of patients with advanced stage disease. However, a number of false positive results could also occur since levels of CA125 could naturally be elevated during ovulation and may also be elevated due to a range of benign gynecologic causes such as fibroids, endometriosis, and pelvic inflammatory disease among others. CA125 can also be elevated in a variety of cancers other than ovarian such as pancreatic, lung, and breast cancer.[4,5] In addition to CA125, other biomarkers are used routinely in medical practice and these include CA 19-9, CA 15-3, CA 72-4, and CEA. CA 19-9 has the advantage of high sensitivity for mucinous ovarian cancers that fail to express CA125.[6] Serum levels of CA 19-9 are elevated in 68% to 83% of mucinous ovarian cancers but in only 28% to 29% of nonmucinous types. CA 15–3, CA 72–4, and CEA levels are found to be elevated in 50% to 56%, 63% to 71%, and 25% to 50% of patients with ovarian cancer.[7,8] However, serial measurement of these tumor markers still plays a vital role in the management of patients with a CA125 negative tumor.[6] Panels of biomarkers are thought to offer the potential for higher discriminatory power. Recent studies that constructed putative biomarker panels with samples from the prostate, lung, colorectal, and ovarian (PLCO) cancer trial found no improvement in diagnostic power in preclinical samples.[9,10] Therefore, researchers still try hard to discover new biomarker to assist diagnose ovarian cancer earlier and can accurately predict the patients' prognosis.

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[a] Department of Obstetrics and Gynecology, [b] Prenatal Diagnosis Center, [c] Outpatient Department of Pediatrics, [d] Department of Gynecological Oncology, The First Hospital of Jilin University, Changchun, China.

[*] Correspondence: Ying Yue, Department of Gynecological Oncology, The First Hospital of Jilin University, Changchun 130021, China (e-mail: yingyue119@126.com).

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High-throughput sequencing is increasingly used and it has been used as a very significant tool for life sciences, such as cancer early diagnosis, cancer stage, and prognosis prediction.[11] In this analysis, we downloaded data from TCGA database and used Edger R package to detect the stage-related differentially expressed genes (DEGs). Followed by, we selected the top 20 hub genes in the 2 groups of DEGs and established PPI network of the stage-related DEGs modules. Moreover, analysis of biological process (BP), molecular function (MF), cellular component (CC), and KEGG pathways of the DEGs and 6 meaningful coexpression modules significantly related to tumor stage were performed. Finally, overall survival (OS) analysis of these genes in the 6 modules was conducted using the Kaplan–Meier plotter online database (http://kmplot.com/analysis/). Then, *COL3A1, COL1A1, COL1A2, KRAS,* and *NRAS* were selected as the prognostic genes that could be used as a new potential biomarker for diagnosis and to predict the prognosis of OC patients.

2. Materials and methods

2.1. Microarray data

Gene expression data of OC patients were downloaded from TCGA database (The Cancer Genome Atlas, http://cancergenome.nih.gov/. The National Cancer Institute and National Human Genome Research Institute work with physicians who collect tissue for TCGA to gain approval with local Institutional Review Boards (IRBs). An IRB is a group of scientists, doctors, clergy, and consumers who review and approve the research proposal for every research project that involves human subjects. These boards ensure that the research is well designed, legal and ethical, and does not involve unnecessary risks to patients.). Meanwhile, the relevant clinical information was also obtained. We download 379 tumor samples of stage II to stage IV. The data is level 3 data which has been normalized.

2.2. Screening of stage-related DEGs and hub genes

We use R Language (Edger R package) to screen the differential genes between ovarian cancer stage II and stage III, and next obtained the DEGs between stage III and stage IV. The adjusted P value $< .05$ and FDR $< .05$ were set as the cut-off criterion. Then, 731DEGs $_{\text{II-III}}$ and 563DEGs $_{\text{III-IV}}$ were detected. CytoHubba is a tool that provides 11 topological analysis methods including Degree, Edge Percolated Component, Maximum Neighborhood Component, Density of Maximum Neighborhood Component, Maximal Clique Centrality, and 6 centralities (Bottleneck, EcCentricity, Closeness, Radiality, Betweenness, and Stress) based on shortest paths to detect the hub genes. Therefore, the top 20 DEGs $_{\text{II-III}}$ hub genes and the top 20 DEGs $_{\text{III-IV}}$ hub genes were screened by the cytoHubba according to the high degree of connectivity, then we selected these DEGs and hub genes between stages for the further analysis.

2.3. Functional and pathway enrichment analysis of DEGs

DAVID is the Database for Annotation, Visualization and Integrated Discovery (https://david.ncifcrf.gov/) which aims to provide online bioinformatics tools for the functional interpretation of lists of genes or proteins.[12] We could visualize the main pathways and biological processes, molecular functions, cellular components among those DEGs through DAVID.

2.4. PPI network, module analysis, and hub genes

Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org) is an online tool designed to evaluate the protein–protein interaction (PPI) information.[13] To look for the potential interaction among those DEGs, we used STRING and input those DEGs into STRING, set confidence score ≥ 0.4, the maximum number of interactors = 0 as the cut-off criterion. Then we used the MCOED to construct 6 correlation modules. The pathway analysis of genes in each module was performed using DAVID. Also, some of the top 20 DEGs $_{\text{II-III}}$ hub genes and the top 20 DEGs $_{\text{III-IV}}$ hub genes which were screened by the cytoHubba according to the high degree of connectivity were found in these modules.[14]

Table 1

Top 20 hub genes in differential genes between stage II and stage III as well as top 20 hub genes in differential genes between stage III and stage IV.

Top 20 hub genes in DEGs II–III		Top 20 hub genes in DEGs III–IV	
Database identifier	Display name	Database identifier	Display name
9606.ENSP00000355645	ACTA1	9606.ENSP00000355645	ACTA1
9606.ENSP00000224784	ACTA2	9606.ENSP00000290378	ACTC1
9606.ENSP00000290378	ACTC1	9606.ENSP00000297323	ADCY1
9606.ENSP00000264705	CAD	9606.ENSP00000295897	ALB
9606.ENSP00000225964	COL1A1	9606.ENSP00000361151	CEL
9606.ENSP00000297268	COL1A2	9606.ENSP00000387699	CREB1
9606.ENSP00000304408	COL3A1	9606.ENSP00000321797	FGF8
9606.ENSP00000052754	DCN	9606.ENSP00000265643	GAL
9606.ENSP00000265171	EGF	9606.ENSP00000331358	GAST
9606.ENSP00000302665	IGF1	9606.ENSP00000240652	IAPP
9606.ENSP00000256078	KRAS	9606.ENSP00000256078	KRAS
9606.ENSP00000266718	LUM	9606.ENSP00000348634	MYH6
9606.ENSP00000215832	MAPK1	9606.ENSP00000366347	NKX2-2
9606.ENSP00000218388	TIMP1	9606.ENSP00000324248	PENK
9606.ENSP00000358548	NRAS	9606.ENSP00000296029	PF4
9606.ENSP00000264380	PIKFYVE	9606.ENSP00000264708	POMC
9606.ENSP00000363092	PRKG1	9606.ENSP00000356438	PTGS2
9606.ENSP00000253788	RPL27	9606.ENSP00000353198	PYY
9606.ENSP00000345957	RPS21	9606.ENSP00000321106	TAC1
9606.ENSP00000361125	VEGFA	9606.ENSP00000284523	WNT3A

2.5. Survival analysis of hub genes in stage-related modules

The Kaplan–Meier plotter (http://kmplot.com/analysis/) is capable of assessing the effect of 54,675 genes on survival using 10,461 cancer samples. These include 5143 breast, 1816 ovarian, 2437 lung, and 1065 gastric cancer patients with a mean follow-up of 69/ 40/ 49/ 33 months. The primary purpose of the tool is a meta-analysis-based biomarker assessment.[15] We used this online tool to plot the OS of the hub genes in the stage-related modules.

2.6. Visualization of the prognostic genes expression level

The Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/index.html) is an interactive web server for analyzing the RNA expression sequencing data of 9736 normal and 8587 tumors samples from the GTEx and TCGA the projects, based on a standard processing pipeline.[16] We displayed the expression level of prognostic genes among stages through the stage plot.

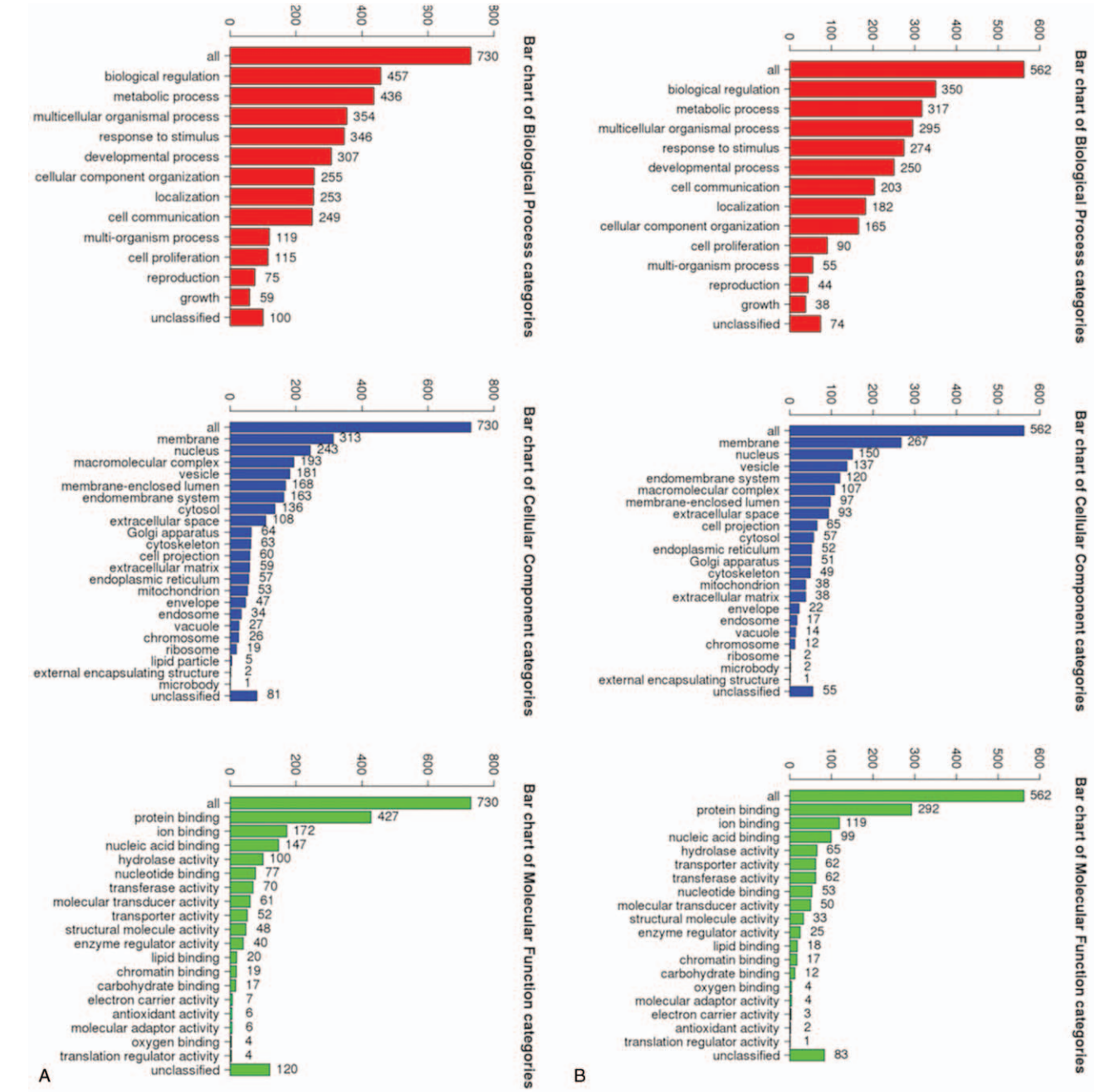


Figure 1. The BP, CC, MF of DEGs $_{II—III}$ (A), and DEGs $_{III-IV}$ (B). BP = biological process, CC = cellular component, MF = molecular function, DEGs $_{II–III}$ = differential genes between ovarian cancer stage II and stage III, DEGs $_{III–IV}$ = differential genes between ovarian cancer stage III and stage IV.

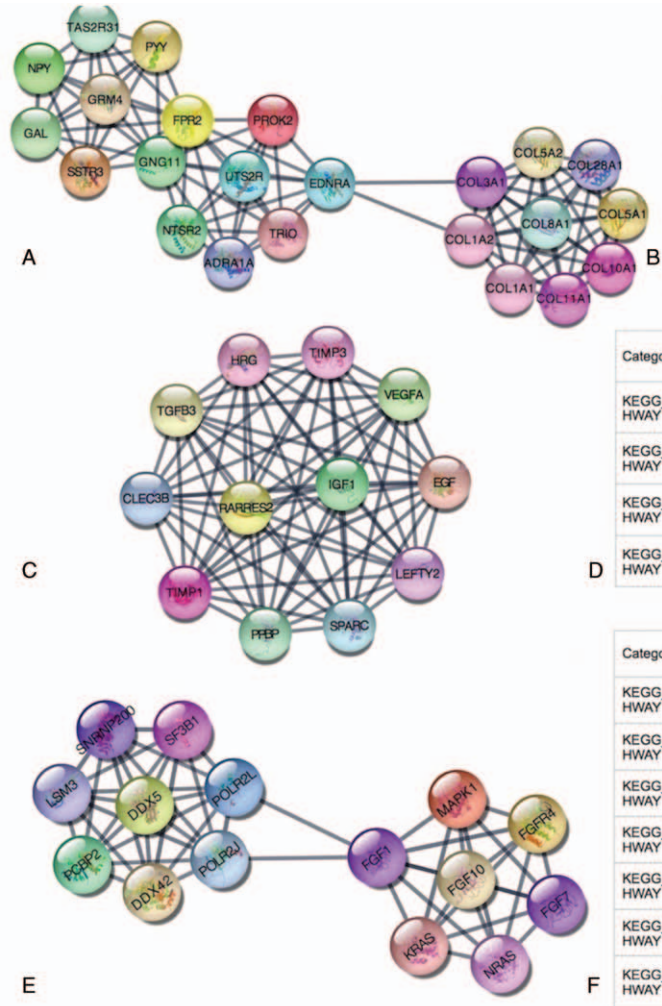
3. Results

3.1. Screening stage-related DEGs and the top 20 hub genes

There were 379 ovarian cancer samples obtained from TCGA in this study. We aimed to obtain the differential genes between stages and found the prognostic genes for ovarian cancer. The R analysis (Edger R package) was applied to detect the DEGs between stage II and stage III, stage III and stage IV, using adjusted P value $< .05$ and FDR < 0.05 as cut-off criteria. Finally, 731 DEGs $_{II\text{-}III}$ that include 328 upregulated and 403 dysregulated genes and 563DEGs $_{III\text{-}IV}$ that include 222 upregulated and 341 dysregulated genes were found. Meanwhile, there are 62 overlap genes between DEGs II-III and DEG III-IV. Moreover, the top 20 DEGs $_{II\text{-}III}$ hub genes and the top 20 DEGs $_{III\text{-}IV}$ hub genes were screened by the cytoHubba according to the high degree of connectivity (Table 1). We selected these DEGs and hub genes between stages for further analysis.

3.2. GO function and KEGG pathway enrichment analysis

For a more profound exploring of the screened DEGs, we used DAVID tools to perform GO function and KEGG pathway enrichment analysis. Above all, we mapped 731 DEGs $_{II\text{-}III}$ and 563 DEGs $_{III\text{-}IV}$ to DAVID web server and conducted GO analysis, the results demonstrated the enriched KEGG pathway of the DEGs $_{II\text{-}III}$ and DEGs $_{III\text{-}IV}$. The DEGs $_{II\text{-}III}$ were most significantly enriched in protein digestion and absorption, ribosome, melanoma, TGF-beta signaling pathway, vascular smooth muscle contraction, signaling pathways regulating pluripotency of stem cells, focal adhesion, platelet activation, malaria, oxytocin signaling pathway. The DEGs $_{III\text{-}IV}$ were mainly enriched in protein digestion and absorption, maturity onset diabetes of the young, ABC transporters, neuroactive ligand-receptor interaction, adrenergic signaling in cardiomyocytes, bile secretion, gap junction, nicotine addiction, pancreatic secretion, melanogenesis. In addition, the BP of DEGs $_{II\text{-}III}$ includes regulation of cell proliferation, tube development, homeostatic process, blood vessel development, response to estrogen stimulus, and cell–cell signaling. For MF, these genes were enriched in growth factor activity, carbohydrate binding, structural molecule activity, heparin binding, and cytokine activity. GO CC analysis also revealed that the DEGs $_{II\text{-}III}$ were significantly enriched in the extracellular region, extracellular region part, proteinaceous extracellular matrix, ribonucleoprotein complex, and cytoplasmic membrane-bounded vesicle



D

Category	Term	Count	%	PValue	Genes
KEGG_PATHWAY	hsa04512:ECM-receptor interaction	7	2.81124498	1.19E-07	CD36, COL3A1, COL1A2, COL1A1, COL5A2, COL11A1, COL5A1
KEGG_PATHWAY	hsa04080:Neuroactive ligand-receptor	7	2.81124498	8.02E-05	EDNRA, GRM4, SSTR3, ADRA1A,
KEGG_PATHWAY	hsa04510:Focal adhesion	6	2.409638554	2.81E-04	COL3A1, COL1A2, COL1A1, COL5A2, COL11A1, COL5A1

Category	Term	Count	%	PValue	Genes
KEGG_PATHWAY	cfa04066:HIF-1 signaling pathway	4	0.22688599	1.11E-04	VEGFA, IGF1, EGF, TIMP1
KEGG_PATHWAY	cfa04060:Cytokine-cytokine receptor	4	0.22688599	0.001014719	PPBP, VEGFA, TGFB3, EGF
KEGG_PATHWAY	cfa05212:Pancreatic cancer	3	0.170164492	0.001786296	VEGFA, TGFB3, EGF
KEGG_PATHWAY	cfa05200:Pathways in cancer	4	0.22688599	0.005633977	VEGFA, TGFB3, IGF1, EGF

F

Category	Term	Count	%	PValue	Genes
KEGG_PATHWAY	chx04015:Rap1 signaling pathway	7	0.27173913	3.21E-07	MAPK1, NRAS, FGFR4, KRAS, FGF7, FGF10, FGF1
KEGG_PATHWAY	chx04810:Regulation of actin cytoskeleton	7	0.27173913	5.22E-07	MAPK1, NRAS, FGFR4, KRAS, FGF7, FGF10, FGF1
KEGG_PATHWAY	chx04014:Ras signaling pathway	7	0.27173913	5.36E-07	MAPK1, NRAS, FGFR4, KRAS, FGF7, FGF10, FGF1
KEGG_PATHWAY	chx04010:MAPK signaling pathway	7	0.27173913	1.10E-06	MAPK1, NRAS, FGFR4, KRAS, FGF7, FGF10, FGF1
KEGG_PATHWAY	chx04151:PI3K-Akt signaling pathway	7	0.27173913	8.94E-06	MAPK1, NRAS, FGFR4, KRAS, FGF7, FGF10, FGF1
KEGG_PATHWAY	chx03040:Spliceosome	5	0.194099379	4.52E-05	SF3B1, SNRNP200, LSM3, DDX5, DDX42
KEGG_PATHWAY	chx05200:Pathways in cancer	6	0.232919255	2.67E-04	MAPK1, NRAS, KRAS, FGF7, FGF10, FGF1

Figure 2. Top 3 modules from the DEGs $_{II–III}$ protein-protein interaction network and the enriched pathways of DEGs $_{II–III}$ modules. DEGs $_{II–III}$ = differential genes between ovarian cancer stage II and stage III, DEGs $_{III–IV}$ = differential genes between ovarian cancer stage III and stage IV.

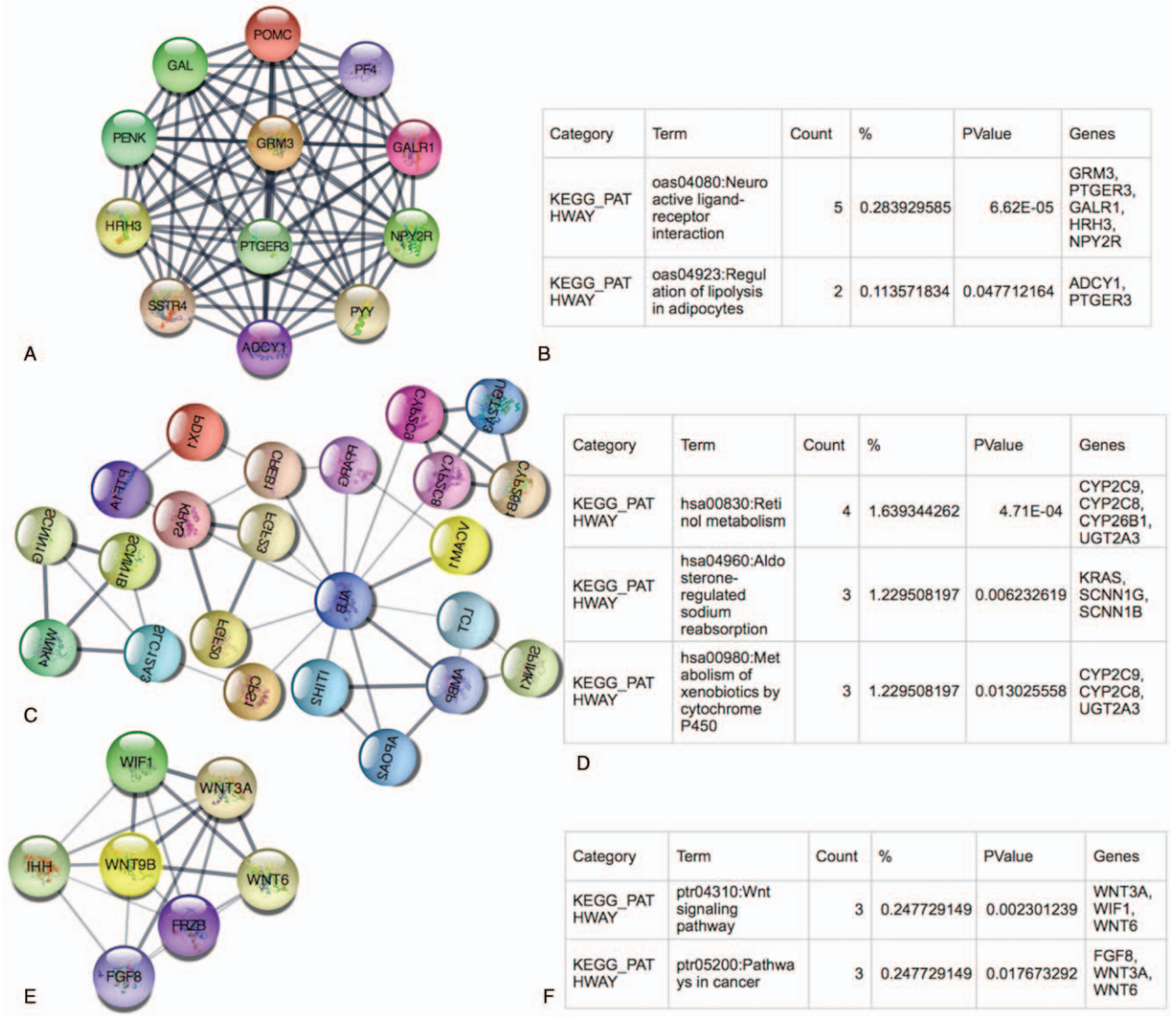
(Fig. 1A). Meanwhile, the BP of DEGs $_{III-IV}$ includes chemical synaptic transmission, cell–cell signaling, positive regulation of cell proliferation, transmembrane transport, cell adhesion. For MF, these genes were enriched in sequence-specific DNA binding, structural constituent of cytoskeleton, heparin binding, transporter activity, and receptor binding. At last GO CC analysis also showed that the DEGs $_{III-IV}$ were significantly enriched in the extracellular space, proteinaceous extracellular matrix, extracellular region, anchored component of membrane (Fig. 1B).

3.3. Construct stage-related EDGs modules from PPI co-expression network

We made the PPI network of these DEGs that based on the information in the STRING protein query from public databases. Aim to detect meaningful modules in this PPI coexpression network, we used MCODE plug-in. The modules of DEGs $_{II-III}$ and DEGs $_{III-IV}$ were conducted separately. KEGG pathway enrichment analysis demonstrated that these 3 DEGs $_{II-III}$ modules were mainly associated with RAS signaling pathway, MAPK signaling pathway, PI3K-Akt signaling pathway, and ECM-receptor interaction, focal adhesion (Fig. 2). Meanwhile, these 3 DEGs $_{III-IV}$ modules were mainly associated with neuroactive ligand-receptor interaction, pathways in cancer, retinol metabolism, WNT signaling pathway (Fig. 3).

3.4. The Kaplan–Meier plotter and expression level of prognostic genes

There are 10 hub genes in the 3 stage-related modules of DEGs $_{II-III}$, they are *COL1A1*, *COL1A2*, *COL3A1*, *EGF*, *IGF1*, *KRAS*, *MAPK1*, *NRAS*, *TIMP1*, *VEGFA*. Besides, there are also 10 hub genes in the 3 stage-related DEGs $_{III-IV}$ modules, they are *ADCY1*, *ALB*, *FGF8*, *GAL*, *KRAS*, *PENK*, *PF4*, *POMC*, *PYY*, *WNT3A*. The prognostic information of the DEGs in these modules was obtained from Kaplan–Meier plotter software (http://kmplot.



Category	Term	Count	%	PValue	Genes
KEGG_PATHWAY	oas04080:Neuroactive ligand-receptor interaction	5	0.283929585	6.62E-05	GRM3, PTGER3, GALR1, HRH3, NPY2R
KEGG_PATHWAY	oas04923:Regulation of lipolysis in adipocytes	2	0.113571834	0.047712164	ADCY1, PTGER3

Category	Term	Count	%	PValue	Genes
KEGG_PATHWAY	hsa00830:Retinol metabolism	4	1.639344262	4.71E-04	CYP2C9, CYP2C8, CYP26B1, UGT2A3
KEGG_PATHWAY	hsa04960:Aldosterone-regulated sodium reabsorption	3	1.229508197	0.006232619	KRAS, SCNN1G, SCNN1B
KEGG_PATHWAY	hsa00980:Metabolism of xenobiotics by cytochrome P450	3	1.229508197	0.013025558	CYP2C9, CYP2C8, UGT2A3

Category	Term	Count	%	PValue	Genes
KEGG_PATHWAY	ptr04310:Wnt signaling pathway	3	0.247729149	0.002301239	WNT3A, WIF1, WNT6
KEGG_PATHWAY	ptr05200:Pathways in cancer	3	0.247729149	0.017673292	FGF8, WNT3A, WNT6

Figure 3. Top 3 modules from the DEGs $_{III-IV}$ protein-protein interaction network and the enriched pathways of DEGs $_{III-IV}$ modules. DEGs $_{II-III}$ = differential genes between ovarian cancer stage II and stage III, DEGs $_{III-IV}$ = differential genes between ovarian cancer stage III and stage IV.

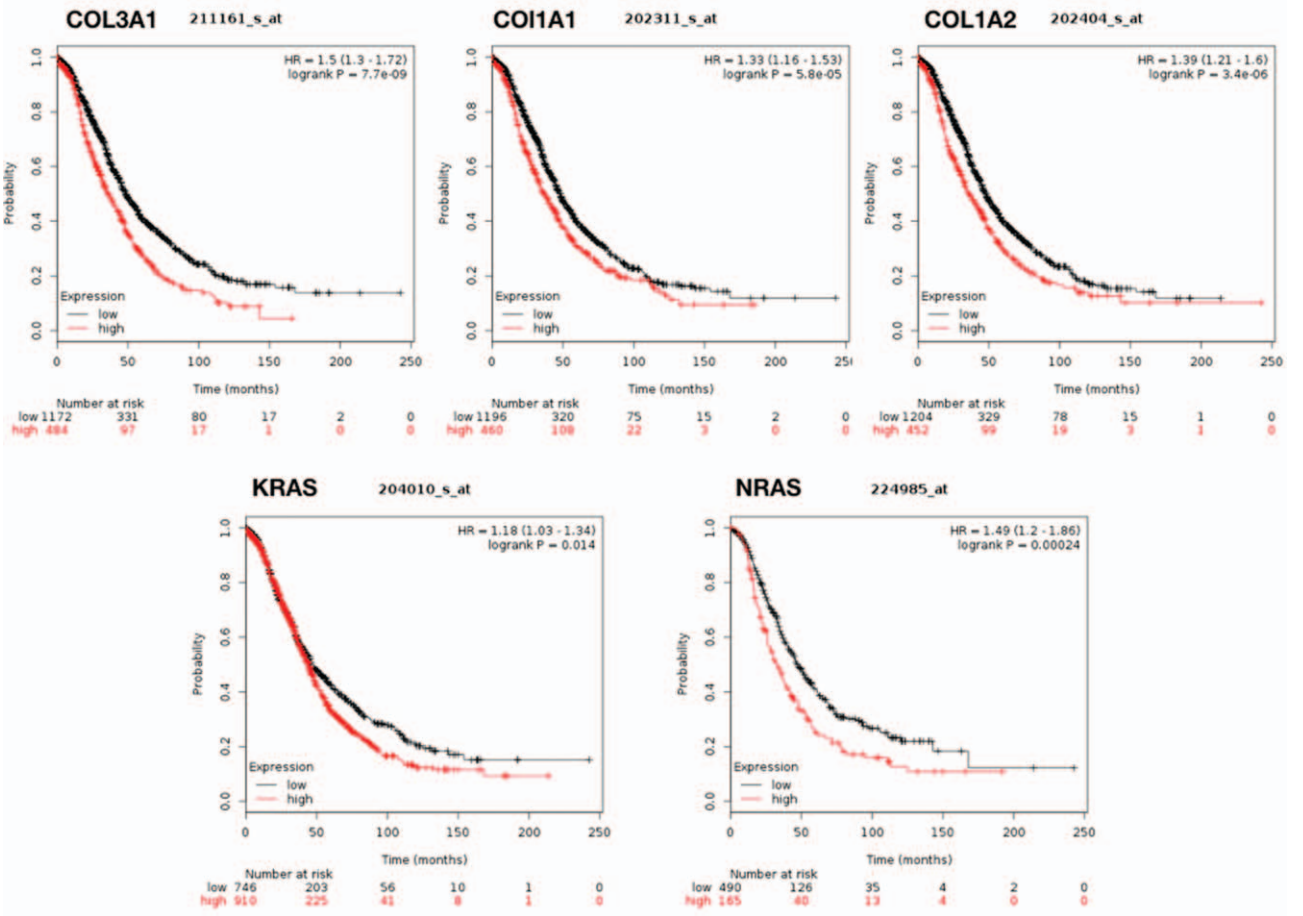


Figure 4. Prognostic value of 5 genes (*COL3A1*, *COL1A2*, *COL1A1*, *KRAS*, *NRAS*) in ovarian cancer patients. HR = hazard ratio, CI = confidence interval.

com/analysis/). It revealed that the expression of *COL3A1* (HR 1.5 [1.3–1.72], $P = 7.7 \times 10–9$) was associated with worse OS for ovarian cancer patients in these DEGs $_{\text{II-III}}$ module as well as *COL1A2* (HR [1.39 1.21–1.6], $P = 3.4 \times 10–6$), *COL1A1* (HR [1.33 1.16–1.53], $P = 5.8 \times 10–5$), *KRAS* (HR 1.18 [1.03–1.34], $P = .014$), *NRAS* (HR 1.49 [1.2–1.86] $P = .00024$). And in DEGs $_{\text{III-IV}}$ modules the expression of *KARS* (HR 1.18 [1.03–1.34], $P = .014$) was associated with worse OS (Fig. 4). Furthermore, we used GEPIA to detect the *COL3A1*, *COL1A1, COL1A2, KRAS, NRAS* expression level in different stages (Fig. 5).

4. Discussion

In this study, a total of 731 DEGs $_{\text{II-III}}$ and 563 DEGs $_{\text{III-IV}}$ were screened, then we conducted 3 DEGs $_{\text{II-III}}$ coexpression modules and 3 DEGs $_{\text{III-IV}}$ coexpression modules. The DEGs $_{\text{II-III}}$ modules were mainly associated with RAS signaling pathway, MAPK signaling pathway, PI3K-Akt signaling pathway, ECM-receptor interaction, and focal adhesion. These pathways might associate with the ovarian cancer progression to the advanced stage. Moreover, *COL3A1, COL1A1, COL1A2, KRAS, NRAS* were selected as the prognostic genes because they are differential genes between the early and advanced cancer stages and might play an unfavorable role in the development of even the metastasis of ovarian cancer. The DEGs $_{\text{III-IV}}$ modules were mainly associated with the neuroactive ligand-receptor interaction, pathways in cancer, retinol metabolism, and WNT signaling pathway, and these pathways might contribute to metastasis of ovarian cancer. All these pathways might function in the progression and metastasis of ovarian cancer. For *KRAS* is the differential gene in both DEGs $_{\text{II-III}}$ module and DEGs $_{\text{III-IV}}$ module, then we select it as a prognostic gene for its worse overall survival of ovarian cancer patients.

Analysis of the 3 selected DEGs $_{\text{II-III}}$ modules from the PPI network showed that advanced ovarian cancer was associated with focal adhesion, PI3K-Akt signaling pathway and ECM-receptor interaction, RAS signaling pathway, MAPK signaling pathway. *COL1A1, COL1A2, COL3A1*were enriched in focal adhesion (bta04510) and ECM-receptor interaction (bta04512) KEGG pathway. It has been shown that ECM containing a large amount of collagen increases the invasiveness and the progression of tumors.[17] It was reported that high expression of focal adhesion kinase activity was associated with elevated level of fibrosis and poor CD8+T cell infiltration. Focal adhesion kinase inhibition could substantially limit tumor progression and extend the survival time of cancer patients.[18] In contrast to normal tissues where collagen is organized as thin, long wavy fibrils parallel to the epithelial boundary, collagen fibrils in tumor stroma are thicker and shorter.[19] In epithelial ovarian cancer, collagen tracts that are perpendicular to the epithelial boundary have been observed.[20] Thus far, the expression of *COL1A1* and *COL1A2* has been noted in gastric cancer and was positively correlated with the degree of invasion, metastasis, and advanced stages.[21]*COL3A1* is frequently in association with type III

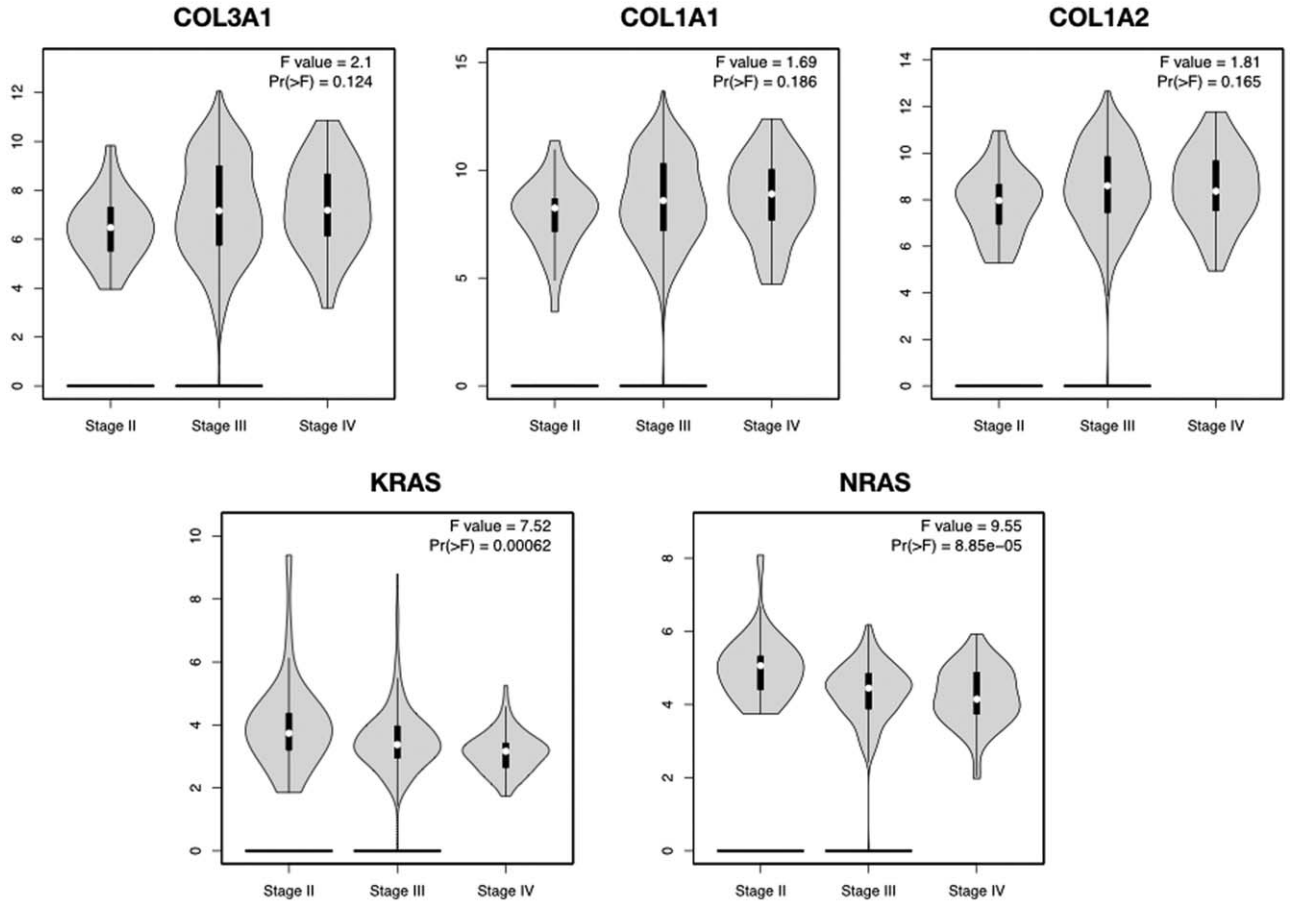


Figure 5. GEPIA database displayed that *COL3A1, COL1A1*, *COL1A2*, *KRAS*, *NRAS* had a strong correlation with the progression of OC based on TCGA data. GEPIA = gene expression profiling interactive analysis, OC = ovarian cancer.

collagen.[22] In our study, *COL3A1* is the differential stage-II-III hub gene and with the worst OS of OC. There was a study demonstrating that *COL3A1* mRNA and protein was upregulated in CRC which was associated with clinicopathologic factors and poor survival, and *COL3A1* was also increased in plasma of CRC patient. *COL3A1* could be a potential diagnostic biomarker for colon cancer.[23] A report indicates that *COL3A1* gene had a prognostic implication in brain tumor.[24] It also was associated with breast cancer development and progression.[25] Moreover, it has been suggested that progressive ovarian carcinoma can induce expression of type III procollagen both in the tumor tissue and peritoneal cavity. In addition, in poorly differentiated serous ovarian carcinoma, the formation of type III procollagen may occur in the neoplastic cells.[26] In serous ovarian carcinoma, production of type III procollagen has been found to be related to an increased degree of malignancy.[26]

KRAS, NRAS are predominantly expressed in various malignancies. RAS has 4 major isoforms, *HRAS, NRAS* and *KRAS* splice variants, *KRas4A* and *KRas4B*.[27,28] RAS protein family members (*KRAS4A, KRAS4B, HRAS,* and *NRAS*) function as GDP-GTP-regulated on-off switches, which regulate cytoplasmic-nuclear signaling networks ruling diverse normal cellular processes. Constitutive activating mutations in RAS genes are found in up to 30% of human cancers,[29] most frequently in *KRAS* (85%), then *NRAS* (15%), then *HRAS* (1%).[30] And remarkably, the oncogenic RAS mutations and mutations in other components of RAS/MAPK signaling pathways seem to be mutually exclusive in most tumors, pointing out that deregulation of RAS-dependent signaling is an essential requirement for tumorigenesis.[29] The mutations of 3 oncogenes *KRAS* and *NRAS* are very important in the development, spread, as well as in diagnostics and therapy of colorectal cancer and melanoma. In addition, *KRAS* and *NRAS* mutations are very well known to be mutated and play important role in pancreatic cancer, lung cancer, bladder cancer, and acute myeloid leukemia.[29] Mucinous ovarian cancer tumors have prevalent *KRAS* mutations. *KRAS* and *NRAS* mutations have been shown to have transforming activity, so it showed that these mutations are rare but important drivers in High grade serous ovarian cystadenocarcinoma (HGS-OvCa).[30]*NRAS* mutations are much rarer than *KRAS*, meta-analysis of colorectal cancer patients carrying *NRAS* mutations had shorter progression-free survival (HR 2.30; 95% CI 1.30–4.07) and shorter overall survival (HR 1.85;95% CI 1.23–2.78).[31] In RAS-driven cancers, *KRas4B* commanded most of the attention. Oncogenic mutants of *KRas4B* are abundant, particularly in adenocarcinomas, appearing in staggering frequencies, although *KRas4A* is currently also reappraised.[32–35] RAS-driven cancer cells predominantly proliferate by the combined action of 2 pathways: MAPK and PI3Kα/Akt.[36] Several studies indicated that numerous components of the phosphatidylinositol-3-kinase (PI3K)/AKT pathway were targeted by amplification, mutation,

and translocation more frequently than any other pathway in cancer patients, leading to pathway activation.

In conclusion, our bioinformatics analysis detected stage-related DEGs and they might play a central role in the development and metastasis of ovarian cancer. In this study, a total of 1294 DEGs and 6 stage-related coexpression network modules were selected, and *COL3A1, COL1A1, COL1A2, KRAS,* and *NRAS* might be the prognostic genes of ovarian cancer. To get more accurate correlation results, we need to make a series of further verification experiments later to prove the results of this prediction.

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Author contributions

YY and LY conceived and designed the experiment. YY, LY, JJ, and LS analyzed data. LY wrote this manuscript. All authors reviewed and approved the final manuscript.
Conceptualization: Lili Yang, Ying Yue.
Data curation: Lili Yang, Liqun Sun.
Formal analysis: Lili Yang, Jili Jing, Liqun Sun.
Investigation: Jili Jing.
Methodology: Lili Yang, Jili Jing, Liqun Sun, Ying Yue.
Resources: Lili Yang.
Software: Lili Yang.
Supervision: Ying Yue.
Validation: Lili Yang, Ying Yue.
Visualization: Lili Yang, Jili Jing.
Writing – original draft: Lili Yang.
Writing – review & editing: Lili Yang, Jili Jing, Liqun Sun, Ying Yue.

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