

Received: 2019.11.24
Accepted: 2019.12.14
Published: 2019.12.24

Identification of Differentially Expressed Genes (DEGs) Relevant to Prognosis of Ovarian Cancer by Use of Integrated Bioinformatics Analysis and Validation by Immunohistochemistry Assay

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: Departmental sources

Background: The aim of this study was to investigate the differentially expressed genes (DEGs) relevant to prognosis of ovarian cancer by use of integrated bioinformatics analysis.





Material/Methods: The DEGs between normal ovary tissue and ovarian cancer tissue were screened in GSE54388, GSE14407, and GSE18520 datasets and the overlapping DEGs were then identified. GO and KEGG enrichment were performed to analyze the biological functions and pathways of the DEGs. A protein-protein interaction (PPI) network of the identified DEGs was constructed using the STRING database. Differences in prognosis between low and high expression of the hub DEGs were also evaluated using the Kaplan-Meier Plotter database. Protein expression of 2 hub genes – BUB1B and KIF201A – was assessed by immunohistochemistry assay and evaluated with the patient's clinical pathology characteristics.

Results: We identified 361 DEGs, mainly involving oncogene-induced cell senescence, cyclin B1-CDK1 complex, protein kinase A catalytic subunit binding, cell cycle, and p53 signaling pathway. Ten hub genes were identified from among the 361 DEGs. The overall survival (OS) and progression-free survival (PFS) of these 10 hub genes were evaluated in the Kaplan-Meier-plotter database. Three (BUB1B, KIF11, and KIF20A) of the 10 hub genes were found to be correlated with ovarian cancer OS and PFS. BUB1B expression level was correlated with ovarian FIGO stage ($p < 0.05$) and tumor differentiation ($p < 0.05$). For KIF20A, the expression level was correlated with FIGO stage ($p < 0.05$) and intraperitoneal metastasis ($p < 0.05$).

Conclusions: DEGs can participate in ovarian cancer development and can be used as biomarkers for prognosis. Patients with upregulated BUB1B, KIF11, and KIF20A tend to have worse overall survival and disease-free survival compared with patients who have low expression.

MeSH Keywords: **Biological Markers • Ovarian Neoplasms • Prognosis**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/921661>

 1540  2  9  30



Background

Ovarian cancer is one of the most frequently diagnosed malignant carcinomas and is the leading cause of female reproductive-related death [1,2]. Early diagnosis of ovarian cancer is difficult due to lack of obvious symptoms and the deep location of tumors in the pelvis. Therefore, many patients miss the best opportunity for surgery due to advanced clinical stage at diagnosis. For early-stage patients who received surgery, tumor recurrence is the leading cause of treatment failure [3–5]. According to the ovarian cancer NCCN guidelines [6], the independent factors relevant to prognosis are clinical stage, tumor differentiation and intraperitoneal metastasis. Recently, some researchers found gene expression profile can also play an important role in prognosis of ovarian cancer patients [7]. Qiu et al. [8] found that Ki-67 was upregulated in ovarian cancer and was obviously correlated with poor prognosis. Sun et al. [9] found that low expression of BCL7A can be used as a biological marker for poor prognosis in ovarian cancer. However, most of the studies relevant to association between gene expression and ovarian cancer patients were mainly focused on 1 or several single genes. It remains unclear whether gene expression profiles differ between the cancer tissue and normal ovary tissue and whether the differently expressed genes played a prognostic role in ovarian cancer. Therefore, in the present study, we screened 3 differentially expressed gene data series to identify the cDEGs and to evaluate the association between DEGs and prognosis of ovarian cancer patients.

Material and Methods

Datasets downloaded

The datasets associated with ovarian cancer were identified from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). Three gene expression datasets – GSE54388 [10], GSE14407 [11], and GSE18520 [12] – were identified and downloaded from the GEO database and were used to detect the differentially expressed genes between ovarian cancer and corresponding normal ovary tissue.

DEGs identification and biological function enrichment

The DEGs between normal ovary tissue and ovarian cancer tissue were first screened in each dataset by the selection criteria of fold change ≥ 2 and p value < 0.05 . The DEGs identified from each dataset were further analyzed to find the genes that overlapped across the 3 datasets. The biological function and pathway of the overlapped DEGs were enriched in the aspects of biological function (BP), cellular component (CC),

molecular function (MF), and KEGG pathway, as demonstrated in a bubble plot.

PPI network construction

A PPI network of the identified 361 DEGs was constructed through the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database under the condition of: (1) minimum required interaction score of 0.4; (2) active interaction sources of text-mining, experiments, databases, co-expression, neighborhood, gene fusion, and co-recurrence; and (3) human species.

Survival analysis

The overall survival (OS) and progression-free survival (PFS) were analyzed based on the Kaplan-Meier Plotter database. The 10 identified hub genes were divided into a low-expression group and a high-expression group according to the median expression level of the tumor tissue. The OS and PFS of the low- and high-expression groups were compared and demonstrated by hazard ratio (HR) and corresponding 95% confidence interval (95%CI).

Immunohistochemistry assay

Fifty patients with confirmed diagnosis of ovarian cancer were included in our study. All patients provided written informed consent, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University, China. The protein expression was assessed by immunohistochemistry assay. The protein expression score was evaluated by 2 pathologists independently according to the following criteria: 0 points: non-staining; 1 points: weak staining (light yellow); 2 points: medium staining (yellow brown); and 3 points: strong staining (brown). The positive rate of tumor cells was as follows: 0 points: no positive tumor cells; 1 point: less than 25% positive tumor cells; 2 points: 25–50% positive tumor cells; 3 points: 50–75% positive tumor cells; 4 points: more than 75% positive tumor cells. The final staining index (SI) was obtained by multiplying the percentage of positive tumor cells with the staining fraction. According to this method, tumors with $SI > 4$ were defined as high expression, $SI < 4$ as low expression, and $SI = 0$ as negative expression.

Statistical analysis

STATA12.0 statistical software was used for data analysis. One-way ANOVA or the t test was used to evaluate differences in expression between cancer tissue and corresponding normal tissues. The log-rank test was applied for survival analysis. A 2-tailed P value of less than 0.5 was deemed as statistically significant.

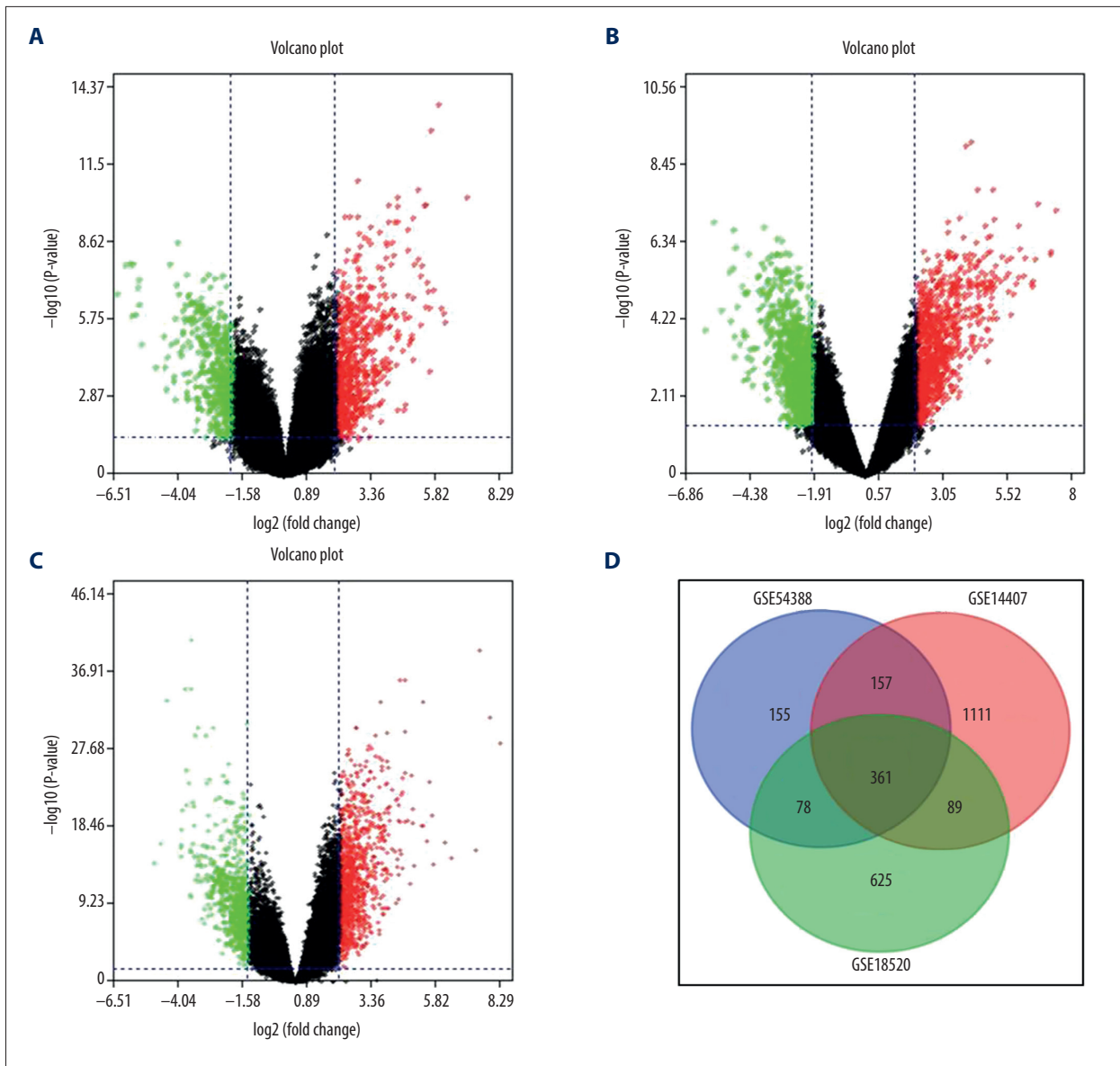


Figure 1. Identification of differentially expressed genes. (A) Volcano plot of of GSE54388; (B) Volcano plot of of GSE14407; (C) Volcano plot of of GSE18520; (D) Venn diagram of the overlapping genes.

Results

DEGs identification

We first screen the DEGs in each data series of GSE54388, GSE14407, and GSE18520 independently. We initially identified 751, 1718, and 1153 DEGs from GSE54388, GSE14407, and GSE18520, respectively, and there were 361 DEGs that overlapped across the 3 gene expression series (Figure 1).

GO and KEGG analysis

Gene ontology (GO) enrichment showed the 361 DEGs mainly involved oncogene-induced cell senescence (Figure 2), cyclin B1-CDK1 complex (Figure 3), and protein kinase A catalytic subunit binding (Figure 4) for the aspects of biological process, cellular component, and molecular function, respectively. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated the 361 DEGs were mainly enriched in cell cycle, p53 signaling pathway, and cellular senescence (Figure 5).

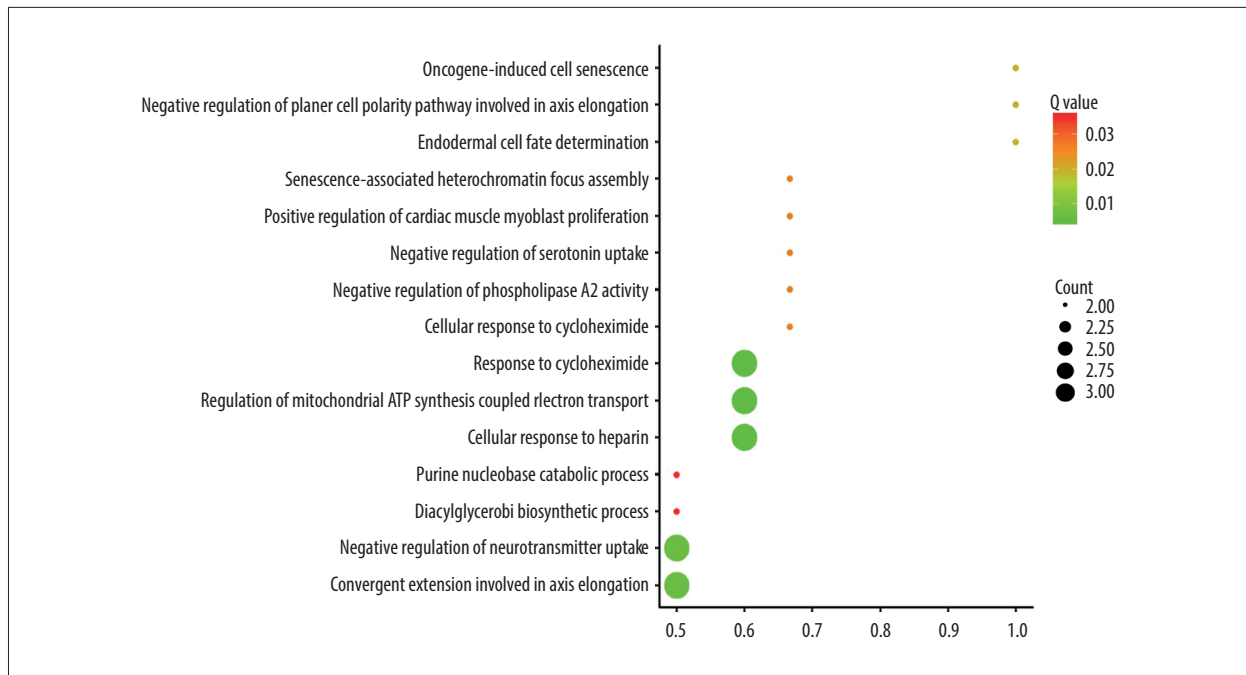


Figure 2. GO analysis of the differentially expressed genes in the aspect of biological process.

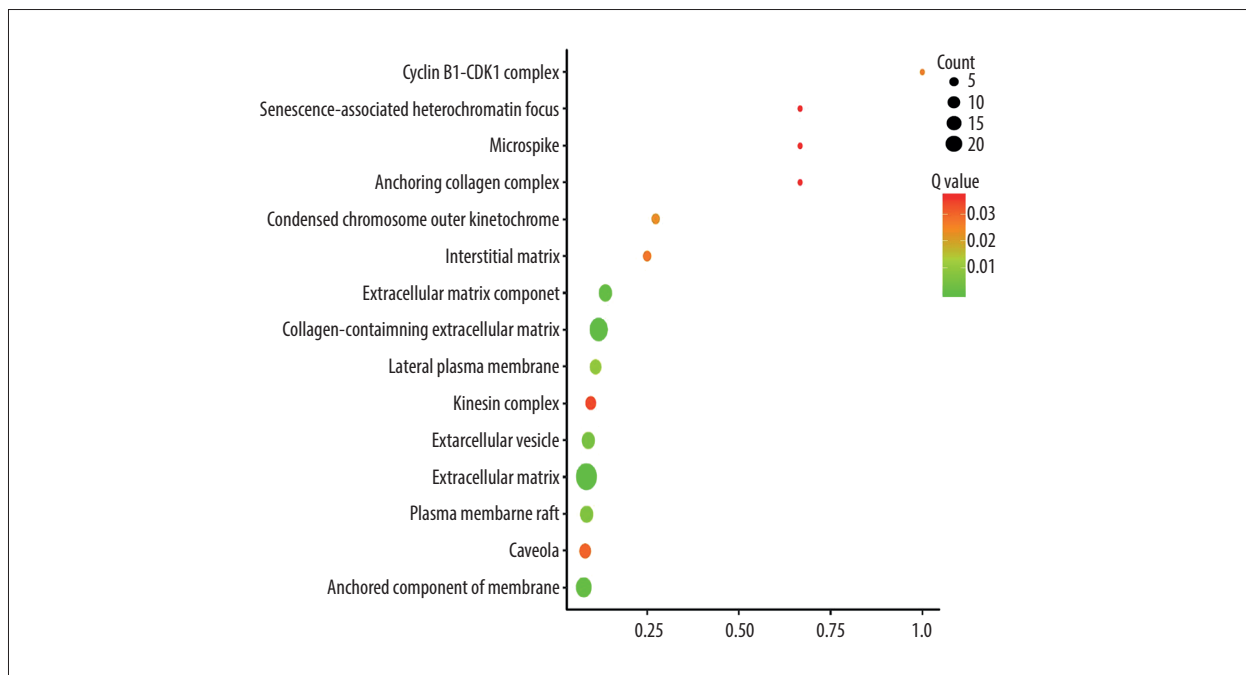


Figure 3. GO analysis of the differentially expressed genes in the aspect of cellular component.

PPI network construction and hub gene identification

A PPI network was constructed using the STRING database. In the network, there were 322 nodes and 1069 edges, with an average node degree of 6.64 and local clustering coefficient of 0.43, which indicated the PPI enrichment was statistically

significant (Figure 6). Ten hub genes were identified from among the 361 DEGs by Cytoscape (Figure 7).

Survival analysis

The OS and PFS of the 10 hub genes were evaluated in the KM PLOTTER database (Table 1). Three hub genes (BUB1B,

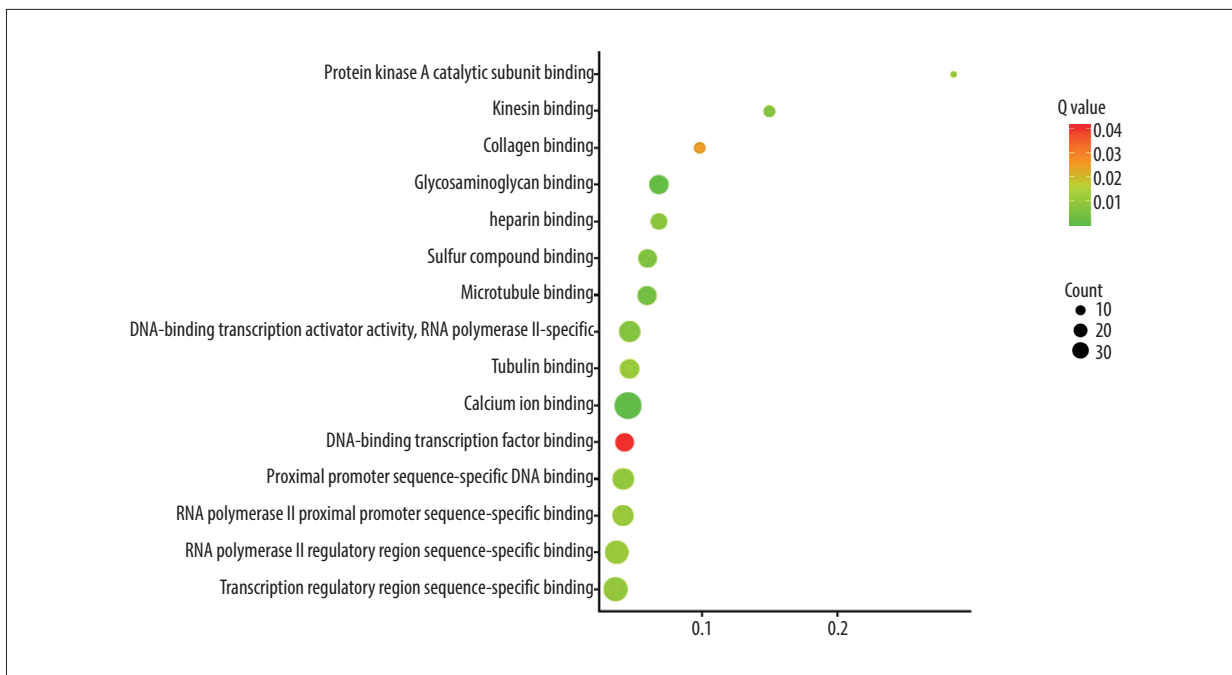


Figure 4. GO analysis of the differentially expressed genes in the aspect of molecular function.

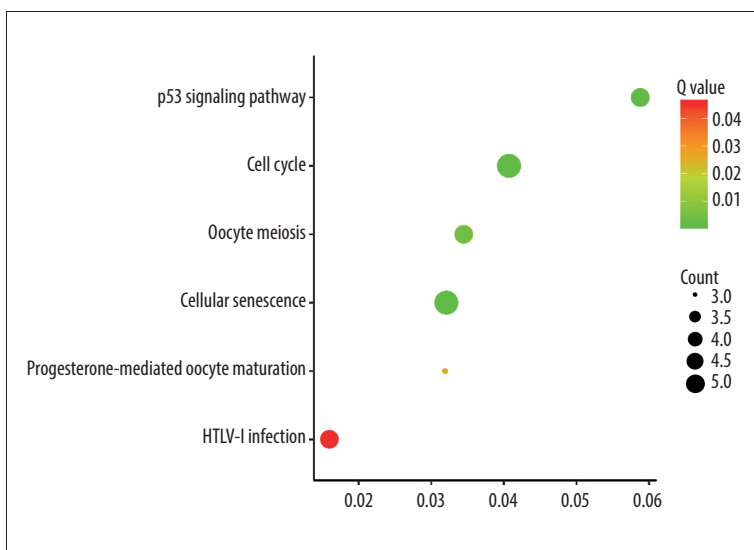


Figure 5. KEGG enrichment of the 361 GGEs.

KIF11, and KIF20A) were identified as being correlated with ovarian cancer overall survival (OS) and progression-free survival (PFS) (Figure 8).

Immunohistochemistry

BUB1B and KIF20A expression was examined by immunohistochemistry assay (Figure 9). Of the 50 included ovarian cancer patients, 24 had high expression of BUB1B and 31 had high expression of KIF20A. The BUB1B expression level was correlated with ovarian FIGO stage ($p < 0.05$) and tumor differentiation ($p < 0.05$). For KIF20A, the expression level was

correlated with FIGO stage ($p < 0.05$) and intraperitoneal metastasis ($p < 0.05$) (Table 2).

Discussion

Epidemiological data show that epithelial ovarian cancer accounts for about 80% of all ovarian malignant tumors [13]. Epithelial ovarian cancer is the leading cause of death in gynecological malignant carcinoma patients in North America, ranking fifth among all cancer-related causes of death [14,15]. An epidemiological study showed that 22 530 people in the

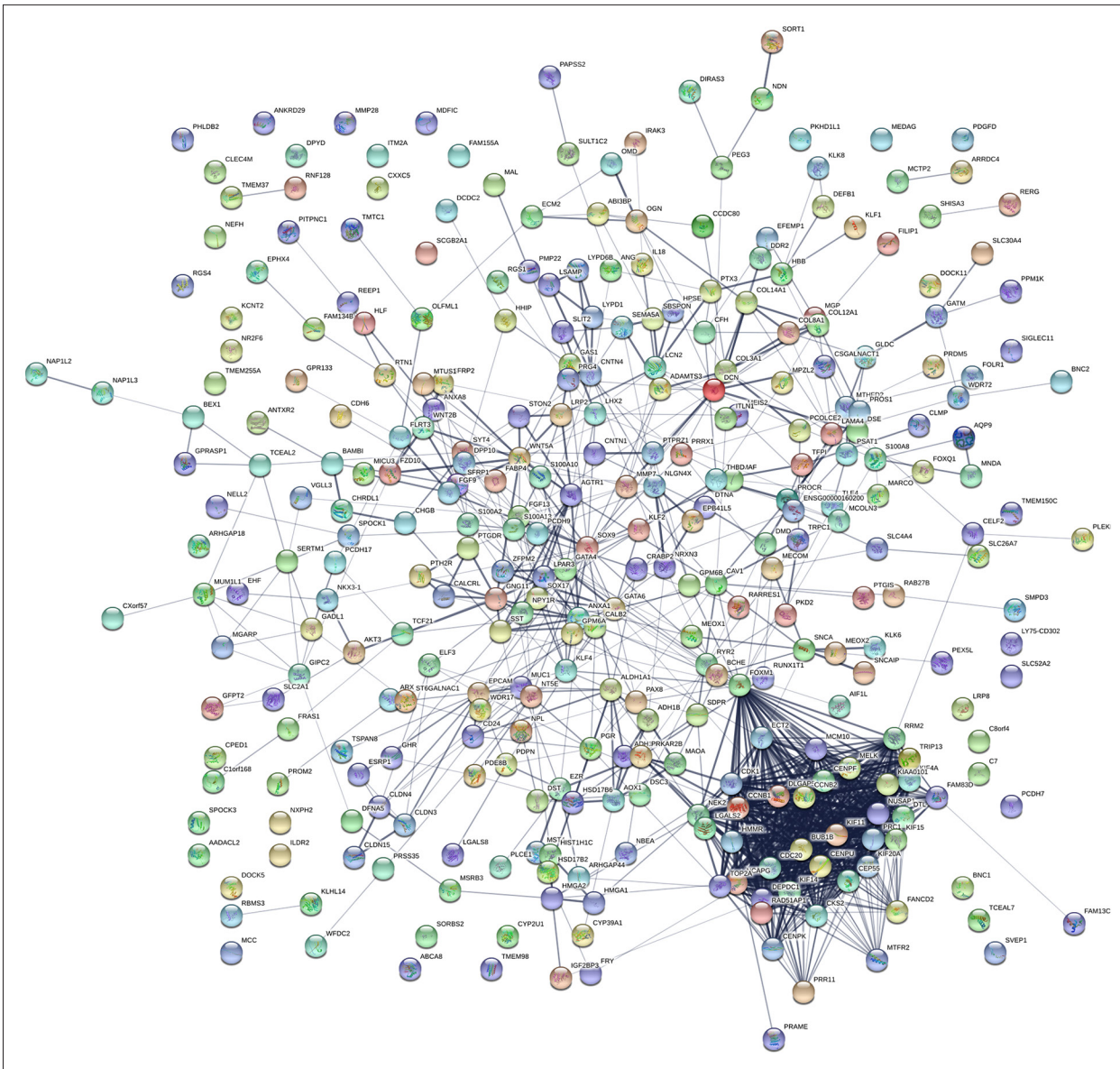


Figure 6. Protein–protein interaction (PPI) network of the dysregulated genes.

United States were diagnosed with ovarian cancer in 2019, while 13 980 people died of ovarian cancer that year, and only about 40% of patients with ovarian cancer could be cured [16]. Therefore, early diagnosis of ovarian cancer is one of the most important factors for prognosis. According to the NCCN guidelines, clinical stage, tumor differentiation, and intraperitoneal metastasis are the independent factors associated with ovarian cancer prognosis [17]. However, in recently years, many studies have also found that dysregulated genes can also play a role in cancer patient prognosis [18–20]. Therefore, it is important to discover the dysregulated genes that are associated with ovarian cancer patient prognosis and to assess their clinical value as biomarkers for predicting patient survival [21,22].

In the present study, we identified 361 DEGs between cancer tissue and normal ovary tissue of ovarian cancer patients through searching 3 different data series (GSE54388, GSE14407, and GSE18520) relevant to ovarian cancer. Of the 361 identified DEGs, 10 hub genes were found to play important roles in ovarian cancer development and to be closely correlated with patient prognosis. Survival analysis also found that BUB1B, KIF11, and KIF20A were correlated with patient OS and DFS. Immunohistochemistry demonstrated that BUB1B expression level was correlated with ovarian FIGO stage ($p < 0.05$) and tumor differentiation ($p < 0.05$). For KIF20A, the expression level was correlated with FIGO stage ($p < 0.05$) and intraperitoneal metastasis ($p < 0.05$). The KIF20A gene is located on human 5q31.2 chromosome and plays an important role in

Table 1. Survival analysis of the 10 hub genes between low- and high-expression groups.

Gene	Overall survival				PFS			
	HR (95% CI)	P value	Median survival (low)	Median survival (high)	HR (95% CI)	P value	Median survival (high)	Median survival (low)
AD51AP1	0.83 (0.66–1.05)	0.12	45.17	44.93	1.11 (0.87–1.42)	0.40	20.07	18.30
CDK1	0.82 (0.65–1.04)	0.10	40.97	48.39	0.77 (0.60–0.99)	0.04	17.07	20.93
NCAPG	1.17 (0.92–1.49)	0.19	45.17	44.13	0.87 (0.69–1.10)	0.25	18.37	19.43
CCNB1	1.35 (1.17–1.57)	4.4E-5	48.00	38.60	1.12 (0.97–1.30)	0.12	20.56	18.10
CCNB2	1.15 (1.00–1.32)	0.05	50.00	43.97	1.11 (0.97–1.25)	0.12	20.53	19.13
CDC20	1.12 (0.99–1.28)	0.08	47.00	41.97	0.93 (0.82–1.05)	0.26	19.00	21.13
BUB1B	1.26 (1.10–1.44)	<0.001	48.06	39.87	1.20 (1.05–1.36)	0.005	20.93	19.00
KIF11	1.24 (1.09–1.43)	0.002	50.00	41.83	1.33 (1.18–1.51)	6.9e-6	22.60	18.00
KIF20A	1.34 (1.14–1.56)	<0.001	52.77	42.58	1.25 (1.09–1.43)	0.002	21.00	19.00
NUSAP1	1.21 (1.06–1.38)	0.004	48.27	43.00	1.12 (0.99–1.27)	0.07	21.00	19.00

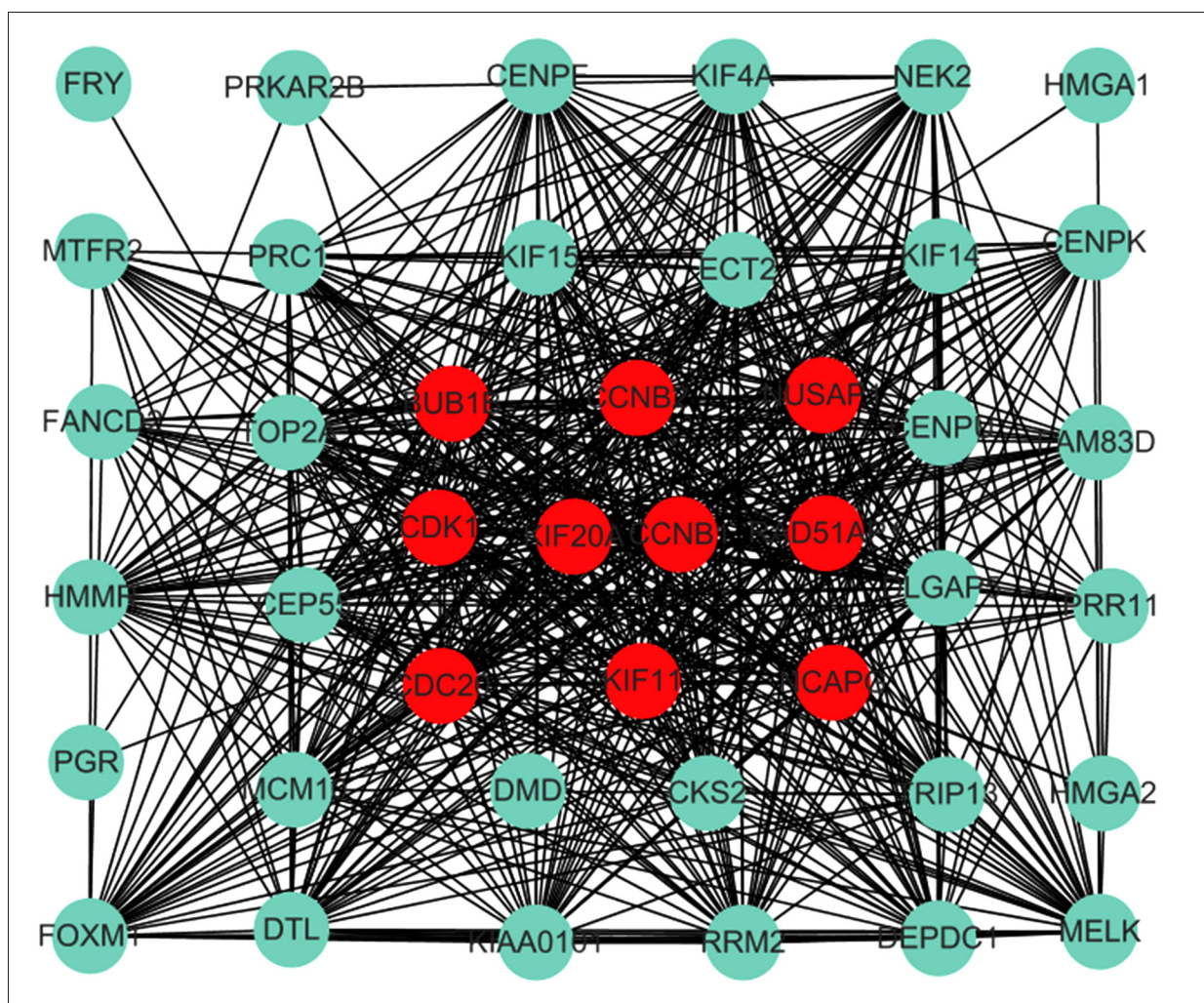


Figure 7. Identified hub genes by CytoHubba among the 361 DEGs.

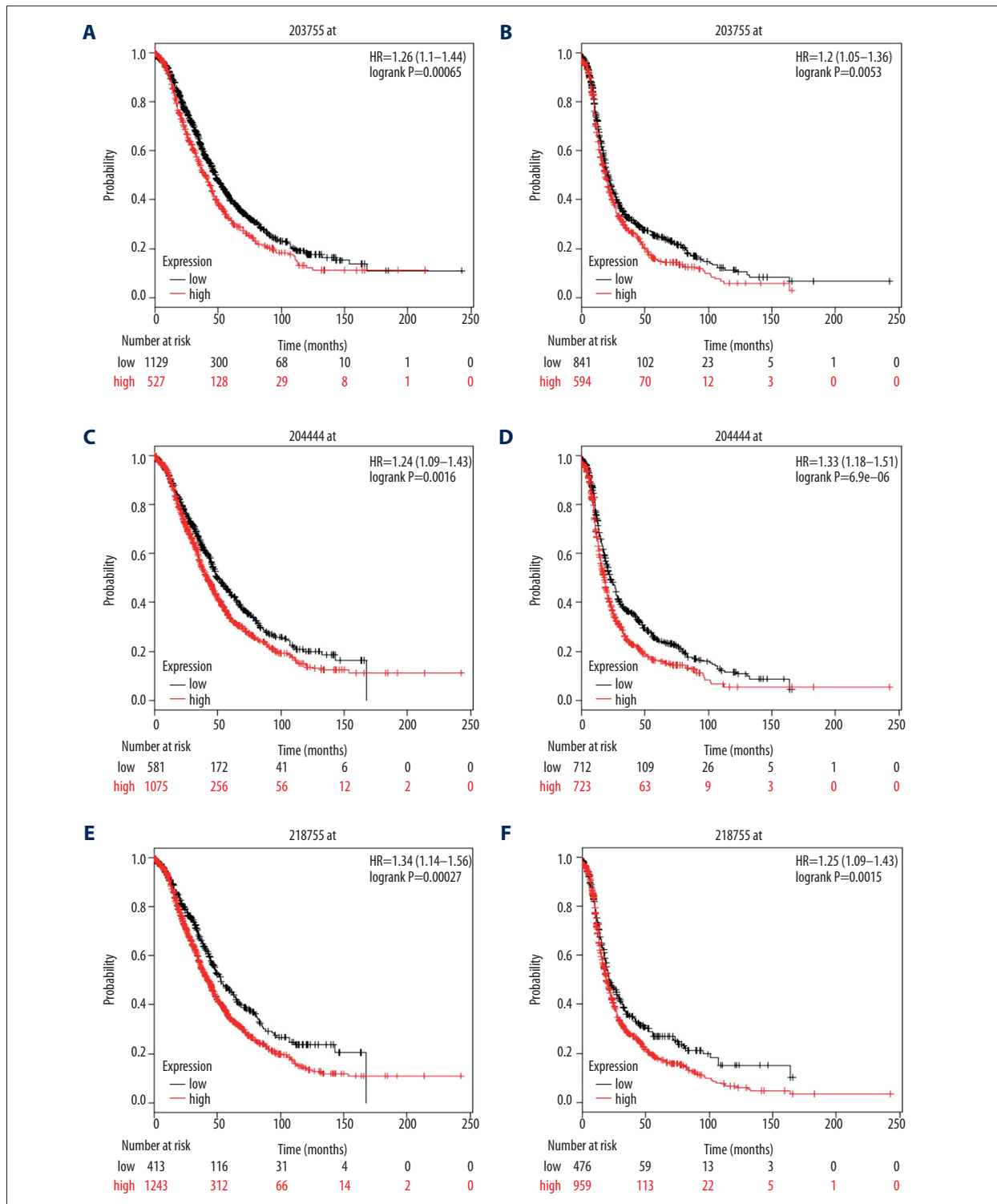


Figure 8. Kaplan-Meier plot of overall survival and progression-free survival for BUB1B, KIF11, and KIF20A high- and low-expression groups. (A) Overall survival compared between BUB1B high- and low-expression groups; (B) Progression-free survival compared between BUB1B high- and low- expression groups; (C) Overall survival comparison compared between KIF11 high- and low-expression groups; (D) Progression-free survival compared between KIF11 high- and low-expression groups; (E) Overall survival comparison compared between KIF20A high- and low-expression groups; (F) Progression-free survival compared between KIF20A high- and low-expression groups.

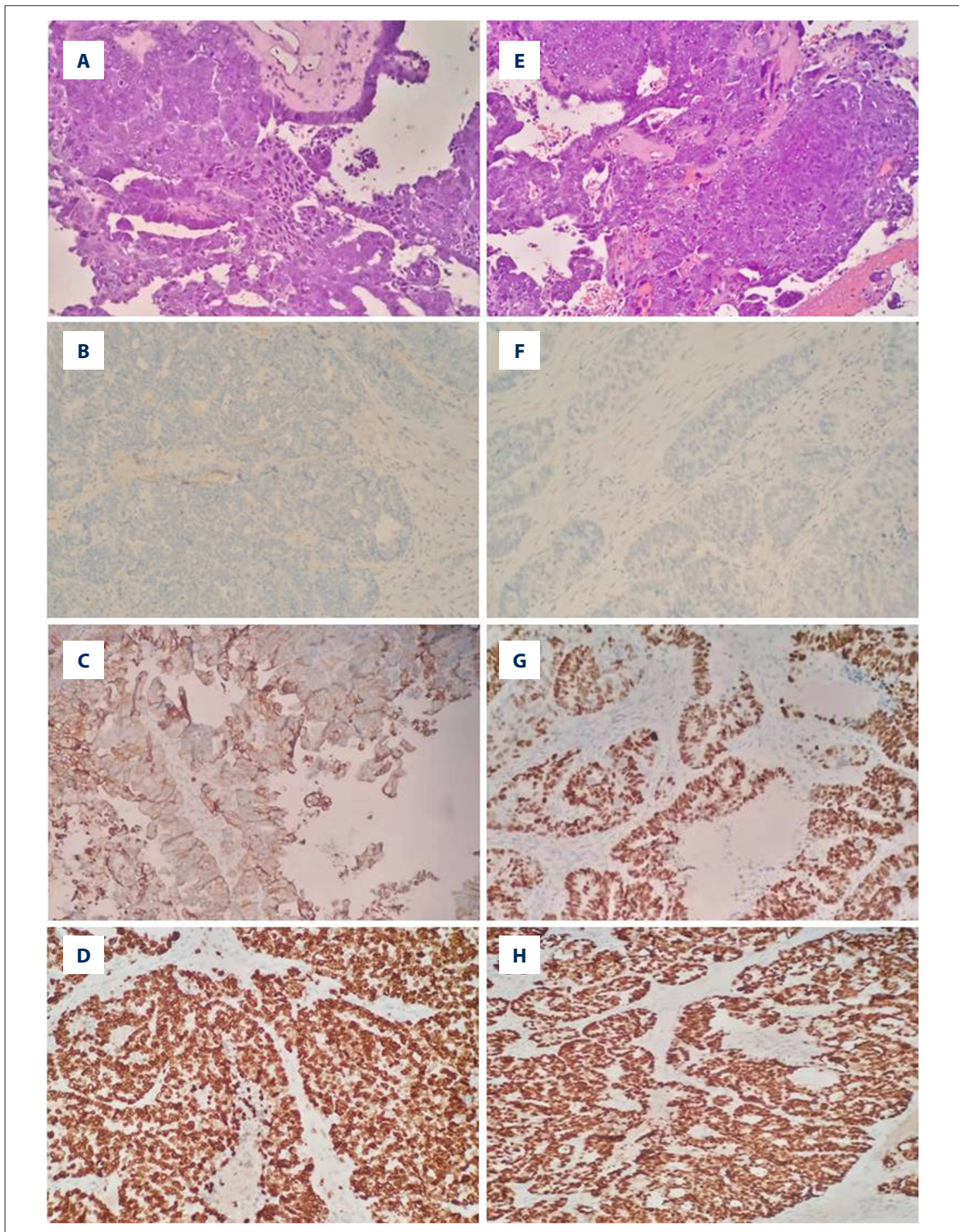


Figure 9. Immunohistochemistry in evaluation BUB1B and KIF20A expression of ovarian cancer. (A) HE staining of ovarian cancer; (B) BUB1B negative expression; (C) BUB1B low expression; (D) BUB1B high expression; (E) HE staining; (F) KIF20A negative expression; (G) KIF20A low expression; (H) KIF20A high expression ($\times 200$).

Table 2. Correlation between BUB1B and KIF20A expression and patient clinical characteristics.

Character	N=50	BUB1B		P	KIF20A		P
		Low (=26)	High (n=24)		Low (n=19)	High=(31)	
Age (years)				>0.05			>0.05
≥50	28	14	14		11	17	
<50	22	12	10		8	14	
FIGO stage				P<0.05			P<0.05
I-II	16	12	4		10	6	
III-IV	34	14	20		9	25	
Pathology type				>0.05			>0.05
Serous adenocarcinoma	22	10	12		8	14	
Mucinous cystadenocarcinoma	18	10	8		7	11	
Ovarian endometrioid carcinoma	10	6	4		4	6	
Differentiation				P<0.05			>0.05
High and moderate	25	14	6				
Low	25	12	18				
Intraperitoneal metastasis				>0.05			P<0.05
No	17	8	9		10	7	
Yes	33	17	15		9	24	
Ca125				>0.05			>0.05
<35 U/mL	8	4	4		3	5	
≥35 U/mL	42	22	20		16	26	

tumorigenesis and development by binding to microtubules, hydrolyzing ATP to produce mechanical energy, and interfering with cell mitosis. Many studies have shown that KIF20A is abnormally expressed in human malignant tumors such as malignant melanoma [6,23], breast cancer [24,25], nasopharyngeal carcinoma [26], pancreatic cancer [27,28], and lung cancer [29], and is closely related to the proliferation, invasion, and prognosis of tumors. However, its correlation with ovarian cancer is unclear. In the present study, we confirmed that ovarian cancer patients with high expression of KIF20A tended to have worse prognosis, overall survival, and progression-free survival. BUB1B, as one of the important proteins in mitotic detection sites, is a multi-domain protein kinase that responds to centromeric tension. It has been found that BUB1B is overexpressed in renal and breast cancer, and its mutation and overexpression are closely related to chromosomal instability [30]. We found that BUB1B was correlated

with ovarian cancer prognosis and can be used as a predictor of poor overall survival and progression-free survival of ovarian cancer patients.

Conclusions

Ovarian cancer is a leading cause of malignant carcinoma-related death for women. DEGs can participate in ovarian cancer development and can be used as biomarkers for prognosis. Patients with high expression of the BUB1B, KIF11, and KIF20A genes tend to have worse overall survival and disease-free survival compared with low-expression patients.

Conflict of interest

None.

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