Research Square

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Screening and identification of key biomarkers and related transcription factors in ovarian cancer by integrated bioinformatics analysis

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Abstract

Background

Ovarian tumors are the most malignant tumors of all gynecological tumors, and although multiple efforts have been made to elucidate the pathogenesis, the molecular mechanisms of ovarian cancer remain unclear.

Methods

In this study, we used bioinformatics to identify genes involved in the carcinogenesis and progression of ovarian cancer. Three microarray datasets (GSE14407, GSE29450, and GSE54388) were downloaded from Gene Expression Omnibus (GEO) database, and differentially expressed genes (DEGs) were identified. For a more in-depth understanding of the DEGs, functional and pathway enrichment analyses were performed and a protein-protein interaction (PPI) network was constructed. The associated transcriptional factor (TFs) regulation network of the DEGs was also constructed. Kaplan Meier-plotter, Gene Expression Profiling Interactive Analysis (GEPIA), the Human Protein Atlas (HPA) database and the Oncomine database were implemented to validated hub genes.

Results

A total of 514 DEGs were detected after the analysis of the three gene expression profiles, including 171 upregulated and 343 downregulated genes. Nine hub genes (CCNB1, CDK1, BUB1, CDC20, CCNA2, BUB1B, AURKA, RRM2, TTK) were obtained from the PPI network. Survival analysis showed that high expression levels of seven hub genes (CCNB1, BUB1, BUB1B, CCNA2, AURKA, CDK1, and RRM2) were associated with worse overall survival (OS). All of seven hub genes were discovered highly expressed in ovarian cancer samples compared to normal ovary samples in GEPIA. Immunostaining results from the HPA database suggested that the expressions of CCNB1, CCNA2, AURKA, and CDK1 proteins were increased in ovarian cancer tissues, and Oncomine analysis indicated that the expression patterns of BUB1B, CCNA2, AURKA, CCNB1, CDK1, and BUB1 have associated with patient clinicopathological information. From the gene-transcriptional factor network, key transcriptional factors, such as POLR2A, ZBTB11, KLF9, and ELF1, were identified with close interactions with these hub genes.

Conclusion

We identified six significant DEGs with poor prognosis in ovarian cancer, which could be of potential biomarkers for ovarian cancer patients.

Background

Among gynecological cancers, the ovarian tumor is one of the three major malignant tumors and the fifth-highest cause of cancerous death among women worldwide (1). Despite advancements in surgical and chemotherapeutic options, the prognosis of ovarian cancer remains poor because of the lack of

effective diagnostic methods at an early stage and the high recurrence rate (2). Thus, studying the molecular mechanisms involved in the genesis and development of ovarian cancer and searching for effective tumor markers is crucial.

In recent years, a large number of bioinformatics studies have been conducted on cancer, suggesting that microarray technology has been widely used to explore the mechanism of cancers (3, 4). Thus, bioinformatics analysis and microarray technology could help us further study and better understand the underlying mechanisms of ovarian cancer. In the current study, three mRNA microarray datasets (GSE14407, GSE29450, and GSE54388) were downloaded from Gene Expression Omnibus (GEO) database. Further, GEO2R online tool and Venn diagram software were applied to obtain common differentially expressed genes (DEGs) between ovarian cancer tissues and non-cancerous tissues from the abovementioned three datasets. Subsequently, gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analyses were performed for evaluating DEGs and protein-protein interaction (PPI) network was constructed. Kaplan Meier-plotter, Gene Expression Profiling Interactive Analysis (GEPIA), the Human Protein Atlas (HPA) database and Oncomine database were implemented to validated hub genes. The associated transcriptional factor (TFs) regulation network of the DEGs was also constructed. In conclusion, 514 DEGs and six hub genes were identified, which may be candidate biomarkers in ovarian cancer.

Material And Methods

Microarray data information

NCBI-GEO (http://www.ncbi.nlm.nih.gov/geo) (5), a free public functional genomics database, contains complete gene expression data, chips, and microarrays. Three gene expression datasets (GSE54388, GSE14407, and GSE29450), containing 16 high-grade ovarian cancer samples and 6 normal ovarian epithelium samples, 12 serous papillary ovarian cancer samples, and 12 normal ovarian epithelium samples, and 10 normal ovarian epithelium samples and 10 clear cell ovarian cancer specimens, were downloaded from GEO (Affymetrix GPL570 platform, Affymetrix Human Genome U133 Plus 2.0 Array).

Identification of DEGs

DEGs between ovarian cancer and non-cancerous samples were screened using the GEO2R online analysis tool (http://www.ncbi.nlm.nih.gov/geo/geo2r). The adjusted $p < 0.01$ and $|logFC| \geq 1$ were set as the cut-off criteria for DEG screening. DEGs with log FC < 0 were considered as downregulated genes, whereas those with log FC > 0 were considered as upregulated genes. The heat-map of DEGs was drawn by the web-based Morpheus software (https://software.broadinstitute.org/morpheus/).

GO and KEGG pathway enrichment analyses of DEGs

To analyze the function of DEGs, GO and KEGG pathway enrichment analyses were conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.ncifcrf.gov) (version 6.8) with $p < 0.05$ as the cut-off criterion.

PPI network and module analysis

Search Tool for the Retrieval of Interacting Genes (STRING; http://string-db.org) (version 11.0), an online tool, was applied to obtain PPI information of DEGs. The PPI network was visualized using Cytoscape, and then the MCODE app in Cytoscape was used to screen modules of PPI network (degree cutoff = 2, node score cut-off = 0.2, k-core = 2, and maximum depth = 100) (6).

Selection and analysis of hub genes

Hub genes were identified from the PPI network using the cytoHubba (Chin et al., 2014) software of Cytoscape. The criterion for selection was degrees ≥ 10. KEGG pathway enrichment was re-analyzed via DAVID ($P < 0.05$) to understand the pathway possibly associated with these hub genes.

Validation of the hub genes

The Kaplan–Meier plotter (http://kmplot.com) assesses the effect of 54,675 genes on survival using 10,188 cancer samples, including samples from 4,142 breast, 1,648 ovarian, 2,437 lung, and 1,065 gastric cancer patients (7). Kaplan–Meier plots were used to analyze overall survival in patients with ovarian cancer. The hazard ratio (HR) with 95% confidence intervals (CIs) and log-rank p values were displayed online on the plot. We used the GEPIA website to analyze the difference in hub gene expression between normal and cancer tissues based on thousands of samples from the GTEx and The Cancer Genome Atlas (TCGA) projects (8). Meanwhile, we also searched and retrieved the HPA data from https://www.proteinatlas.org/, to compare the expression patterns of these genes in ovarian cancer and normal tissues. The HPA database contains immunohistochemical data for protein expression of 17 main cancer types in samples from 8,000 patients.

Oncomine database extraction

The relationship between expression patterns and tumor grades, KRAS mutation status was analyzed using Oncomine database online (http://www.oncomine.com).

Transcriptional factor regulatory network of hub genes

NetworkAnalyst (http://www.networkanalyst.ca/faces/home.xhtml) is a comprehensive web-based platform for network-based visual analytics of gene expression profiling, meta-analysis, and interpretation. In this study, the TFs of the hub genes were predicted from this database and a gene-TF regulatory network was constructed and visualized by the Cytoscape software.

Results

Identification of DEGs in ovarian cancers

DEGs (2685 in GSE14407, 2104 in GSE29450, and 2305 in GSE54388) were identified via GEO2R online tools. Following this, 514 common DEGs were identified from the abovementioned three datasets using

the Venn diagram software (Fig. 1), consisting of 343 downregulated genes and 171 upregulated genes between ovarian cancer tissues and non-cancerous tissues (Fig. 2).

KEGG and GO enrichment analyses

To analyze the function of DEGs, GO function and KEGG pathway enrichment analysis were performed using DAVID.

The GO analysis results (Fig. 3) suggested the following:

1) In terms of biological processes (BPs), 514 DEGs were particularly enriched in cell division, mitotic nuclear division, sister chromatid cohesion, DNA replication, mitotic sister chromatid segregation, cell proliferation, protein localization to kinetochore, extracellular matrix organization, cellular response to transforming growth factor beta stimulus, and G1/S transition of mitotic cell cycle.

2) In terms of molecular function (MF), the DEGs were mainly enriched in protein binding, ATP binding, protein kinase A catalytic subunit binding, oxidoreductase activity, helicase activity, sequence-specific DNA binding, microtubule-binding, heparin-binding, ATP-dependent microtubule motor activity and plus-end-directed.

3) For GO cell component (CC), the DEGs were enriched in cytosol, midbody, condensed chromosome kinetochore, cytoplasm, spindle midzone, spindle pole, spindle microtubule, chromosome passenger complex, kinetochore, and extracellular exosome.

KEGG analysis results, demonstrating that 514 DEGs were particularly enriched in cell cycle, Focal adhesion, PI3K-Akt signaling pathway, Oocyte meiosis, Pathways in cancer and Progesterone-mediated oocyte maturation, are shown in Fig. 4.

PPI network and module analysis

Based on the information from STRING database, with the minimum required interaction score of 0.07, the final PPI network had 477 nodes and 1385 edges, the average aggregation coefficient was 0.365, and the enrichment was $p < 1.0e-16$ (Fig. 5a). The most significant module was obtained using Cytoscape (Fig. 5b).

Hub gene selection and analysis

The criteria for selection of hub genes were degrees ≥ 10, and we screened the top 20 genes according to the degree ranking.

KEGG pathway enrichment was re-analyzed to understand the possible pathways of these 20 selected genes. Results showed that nine genes [Cyclin B1(CCNB1), cyclin dependent kinase 1(CDK1), BUB1 mitotic checkpoint serine/threonine kinase (BUB1), TTK protein kinase (TTK), BUB1 mitotic checkpoint

serine/threonine kinase B (BUB1B), cell division cycle 20(CDC20), Cyclin A2 (CCNA2), aurora kinase A (AURKA), and ribonucleotide reductase regulatory subunit M2 (RRM2)] were particularly enriched in five significant signaling pathways: cell cycle, oocyte meiosis, progesterone-mediated oocyte maturation, p53 signaling pathway, and viral carcinogenesis ($P < 0.05$, Table 1). Thus, these nine genes were identified as the hub genes for further validation.

Table 1
Re-analysis of 20 selected hub genes via KEGG* pathway enrichment

Term	Count	P-value	Genes
cfa04110: Cell cycle	7	2.60E-09	CCNB1,CDK1, BUB1,TTK,BUB1B,CDC20,CCNA2
cfa04914: Progesterone-mediated oocyte maturation	4	1.59E-04	CCNB1,CDK1,BUB1,CCNA2
cfa04114: Oocyte meiosis	4	3.12E-04	CDK1, BUB1, CDC20, AURKA
cfa04115: p53 signaling pathway	3	0.0031471	CCNB1,CDK1,RRM2
cfa05203: Viral carcinogenesis	3	0.025403142	C DK1, CDC20, CCNA2
*KEGG, Kyoto Encyclopedia of Genes and Genomes.			

Meanwhile, We mapped the expression calorimetry of the top 60 common DEGs in three datasets, and the DEGs were clustered into two groups: cancer samples and normal samples (Fig. 6), the heatmap showed that expressions of DEGs differed in normal ovary tissues and ovarian carcinoma tissues.

Validation of the hub genes

Kaplan–Meier plotter (http://kmplot.com/analysis) was used to identify the survival data of the abovementioned nine hub genes. The results suggested that seven of the nine hub genes were associated with overall survival in ovarian cancer patients. High expression of CCNB1 (HR: 1.92 [1.55–2.38], P = 1.1e-09), as well as AURKA (HR: 1.34 [1.18–1.52], P = 9.6e-06), BUB1B (HR: 1.26 [1.1–1.44], P = 0.00065), BUB1 (HR: 1.26 [1.08–1.46], P = 0.0029), CCNA2 (HR: 1.19 [1.03–1.37], P = 0.02), RRM2 (HR: 1.17[1.03–1.34], P = 0.017), and CDK1 (HR: 1.27 (1.11–1.46), P = 6e-04) (Fig. 7), was associated with worse overall survival in ovarian cancer patients, respectively.

The GEPIA webserver was used to analyze the difference in hub gene expression between normal and cancer tissues. The results suggested high expression levels of all of the abovementioned seven genes in the cancerous tissue samples when compared with the normal tissue samples ($P < 0.05$, Fig. 8). Figure 9 showed that apart from RRM2, expression of CCNB1, CCNA2, AURKA, and CDK1 proteins was also higher in ovarian cancer than in normal tissues using the HPA dataset. The HPA lacks data on BUB1 and BUB1B protein. Finally, these six genes (CCNB1, CCNA2, AURKA, BUB1, BUB1B, and CDK1) were identified as the hub genes that are essential for ovarian cancer development.

Hub gene analysis in oncomine database

In the Sieben Ovarian dataset, oncomine analysis results suggested that mRNA levels of AURKA, BUB1B, and CCNA2 were associated with tumor grade (Fig. 10) and mRNA levels of AURKA, BUB1, BUB1B, CDK1, and CCNA2 were associated with KRAS mutation (Fig. 11). However the Sieben Ovarian dataset lacks data on CCNB1, so we analyzed it in the Welsh Ovarian dataset, the result showed that CCNB1 mRNAs from the higher-grade cancer samples displayed higher levels than those from lower-grade cancer tissues (Fig. 10).

Transcriptional factor regulatory network analysis of hub genes

For these six hub genes, the TF-gene regulatory network was constructed including 95 edges and 74 TFs (Fig. 12), In brief, AURKA was regulated by 19 TFs, BUB1 regulated by 18 TFs, CCNA2 regulated by 14 TFs, CCNB1 regulated by 11 TFs, BUB1B regulated by 15 TFs and CDK1 regulated by 18 TFs. Moreover, various TFs were found to regulate more than one hub gene, 17 TFs were identified with a connectivity degree ≥ 2 and 4 TFs were identified with a connectivity degree ≥ 3 in the gene-TF regulatory network (Table 2), which indicated that these TFs have close interactions with the six hub DEGs. For example, Kruppel Like Factor 9 (KLF9) was predicted to regulate AURKA, CCNA2, and CDK1; the RNA polymerase II subunit A (POLR2A) could regulate CCNB1, AURKA, and BUB1.

Table 2
The transcription factors (TFs) of hub genes

TFs	Genes	Count
POLR2A	CCNB1 BUB1 AURKA	3
ZBTB11	CCNA2 BUB1 AURKA	3
ELF1	CCNA2 BUB1 BUB1B	3
KLF9	CCNA2 CDK1 AURKA	3

Discussion

Among gynecologic cancers, ovarian cancer is one of the three major malignancies and the fifth most common cause of cancerous death among women worldwide (1). Despite the rapid development of surgery and medical therapy, the high mortality rate in ovarian cancer has not improved in the past decades. One of the most important reasons for this may be that in most cases, ovarian cancer is not diagnosed at an early stage, and thus cannot be treated promptly. Therefore, it is crucial to study the molecular mechanisms involved in the genesis and development of ovarian cancer and identify effective tumor markers.

Microarray technology enables us to identify crucial genetic alterations in ovarian cancer and has been proved to be a useful tool for identifying new biomarkers for the diagnosis, treatment, and prognosis of ovarian cancer. Using bioinformatics analysis, 514 common DEGs were screened from three mRNA microarray datasets in the current study. PPI network analysis, GO analysis, and KEGG pathway enrichment analysis were performed for a more in-depth study of these DEGs. The KEGG pathway analysis showed that the DEGs were mainly involved in cancer-related pathways, such as the PI3K-Akt signaling pathway, cell cycle, focal adhesion and proteoglycans in cancer. Furthermore, nine hub genes (CCNB1, CCNA2, AURKA, CDK1, BUB1B, RRM2, TTK, CDC20, and BUB1) were further identified.

Following this, survival analysis revealed that high expressions of seven hub genes(CCNB1, CCNA2, AURKA, CDK1, BUB1B, RRM2, and BUB1) were significantly associated with worse overall survival of ovarian cancer patients, which indicated that these seven hub genes may be regarded as prognostic biomarkers for ovarian cancer. we also confirmed and validated transcriptional and translational levels in ovarian cancer of these seven hub genes using GEPIA and HPA datasets, the results indicated that apart from RRM2, CCNB1, CCNA2, AURKA, CDK1 were highly expressed in ovarian cancer. The HPA lacks data on BUB1 and BUB1B protein, but the expression analysis from GEPIA suggested the overexpression of BUB1 and BUB1B in ovarian cancer tissues. Thus, these six genes(CCNB1, CCNA2, AURKA, CDK1, BUB1, and BUB1B) were finally identified as the hub genes involved in the development of ovarian cancer.

Kras, a mouse sarcomatoid virus oncogene, is one of the most frequently mutated oncogenes in cancer. Several studies have reported that the oncogene KRAS plays an important role in ovarian cancer. Jones, et al. suggested that metastatic type 1 ovarian cancer with KRAS mutation has a poor prognosis (9). A recent study indicated that KRAS promoted ovarian cancer dissemination by stabilizing spheroid formation (10). Through oncomine analysis, the current study results suggested that patients with KRAS mutation status displayed higher expression levels of AURKA, BUB1, BUB1B, CDK1 and CCNA2 than those with KRAS wild type status. Furthermore, mRNA levels of AURKA, BUB1B, CCNA2, and CCNB1 were associated with tumor grade, the higher the expression levels of the hub genes, the higher the tumor grade. This result demonstrated that these six hub genes may play a key role in carcinogenesis or progression of ovarian cancer.

BUB1B encodes a kinase participating in the spindle checkpoint, which has been demonstrated to be associated with cancer stem cell tumorigenesis and resistance to radiation (11). In human tumor cells, a reduction in the level of BUB1B or inhibition of BUB1B kinase activity results in the loss of many chromosomes and apoptotic cell death (12). AURKA, a key regulator of cell cycle, is essential for the correct formation of the mitotic spindle. Abnormal AURKA activity usually involves carcinogenic transformation and cancer progression (13). Our results are consistent with those of Kulbe's (14), which reported that AURKA is specifically overexpressed in the malignant epithelium of ovaries and high AURKA expression levels are associated with a poor prognosis. Moreover, compared with the recently reported biomarker fibroblast growth factor 18 (fgf18), AURKA performed better in differentiating between malignant and benign diseases (15). Thus, we have reason to speculate that AURKA has potential as a biomarker in ovarian cancer. CCNA2, which is also an important cell cycle regulatory gene, has been

revealed as a hub gene in other gynecological cancers, such as cervical, endometrial, and vulvar cancers (16, 17). In ovarian cancer, our results suggest that CCNA2 is overexpressed and associated with cancer prognosis, tumor grade, and KRAS mutation, indicating the critical role of CCNA2 in ovarian cancer. In chemo-resistant epithelial ovarian cancer, the study by Woong Ju BCY reported that CCNA2 was also upregulated (18). Cyclin-dependent kinase 1 (CDK1), a core regulator of the G2-M checkpoint, ranked first in the current PPI network. Indeed, CDK1 has been found to be associated with the clinicopathological factors of ovarian cancer in multiple studies(19–21), the results of the current study further support these previous data. Cyclin b1 (CCNB1) is mainly expressed in the G2/M phase and plays an important role in mitosis. Studies have shown that CCNB1 is overexpressed in many cancers and higher levels of CCNB1 mRNA have been associated with poor prognosis (22–24), In 2018, Chong, et al. reported that depletion of spindle checkpoint-related genes BUB1, CCNB1, CENPE, and CENPF during paclitaxel resistance can lead to the suppression of paclitaxel-induced cell death in ovarian cancer (25). BUB1, a serine/threonine kinase, which is encoded by BUBI gene plays a central role in mitosis. A growing body of evidence indicates that BUB1 plays an important role in carcinogenesis and progression of various cancers, including gastric cancer (26), pancreatic ductal adenocarcinoma (27), glioblastoma (28), and hepatocellular carcinoma (29). Moreover, BUB1 has been reported to be important in tumor therapeutic response (25, 30–32); recently, a study confirmed the role of BUB1 in promoting proliferation and IR resistance in glioblastomas (32).

TFs, regulators of gene expression, regulate their expression by binding to the promoter region of target genes, therefore, they are essentially involved in the development and progression of human cancers. In the current study, we constructed The hub gene-TF regulatory network and identified some TFs with close interactions with hub genes. For example, POLR2A was able to regulate the expression of CCNB1, BUB1, and AURKA; ELF1 was able to regulate the expression of CCNA2 BUB1 BUB1B. These TFs formed a connected regulatory network with hub genes, which indicated that they maybe play a role in carcinogenesis or progression of ovarian cancer.

Conclusion

In conclusion, through bioinformatics analysis, six hub genes (BUB1B, CCNA2, AURKA, CDK1, BUB1, and CCNB1) were identified between ovarian cancer tissues and normal ovarian tissues in the current study, which may be regarded as diagnostic and prognostic biomarkers of ovarian cancer. However, further series of experiments are needed to verify the biological functions of these genes in ovarian cancer patients. Nonetheless, the present study provides some useful information to understand the mechanism of the occurrence and development of ovarian tumors.

Abbreviations

The Cancer Genome Atlas: TCGA; Gene Expression Omnibus: GEO; Differentially expressed genes: DEGs; Protein-protein interaction : PPI; Gene Expression Profiling Interactive Analysis: GEPIA; Overall survival: OS; Gene ontology: GO; Kyoto encyclopedia of genes and genomes: KEGG; the Database for Annotation,

Visualization and Integrated Discovery: DAVID; Search Tool for the Retrieval of Interacting Genes: STRING; Hazard ratio: HR; Confidence intervals: Cis; Biological processes: BPs; Molecular function: MF; Cell component: CC; Fold change: FC; transcriptional factor: TF; Human Protein Atlas: HPA.

Declarations

Ethics approval and consent to participate

Not applicable

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Authors'contributions

All authors conceived and designed the research. Qiang Yuan and Wenqiong Qin contributed to the bioinformatics analysis and drafted the manuscript. Wenqiong Qin, Qiang Yuan, Yi Liu, Ying Zeng, Dandan ke, Xiaoyan Dai, and Yu Shuai contributed to the data mining. Jiaqi Hu and Hua Shi contributed to the statistical analysis and modified the manuscript. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the research in ensuring that the accuracy and integrity of any part of the work are appropriately investigated and resolved.

Availability of data and materials

All data generated or analyzed during the present study are included in this article.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

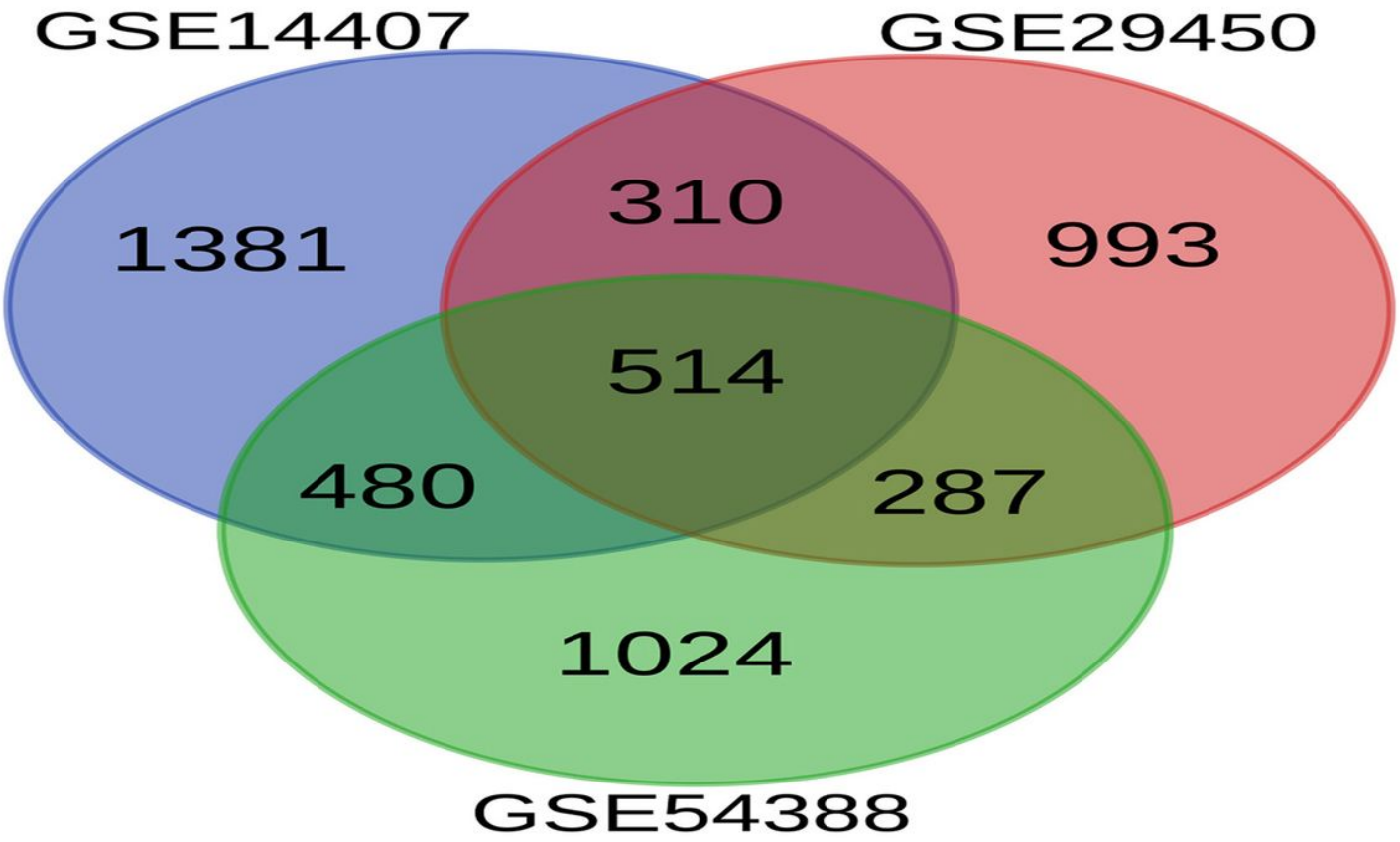

Figure 1

Identification of 514 common DEGs in the three datasets (GSE14407, GSE54388 and GSE29450) through Venn diagrams software (available online: http://bioinformatics.psb.ugent.be/webtools/Venn/). Different colors meant different datasets.

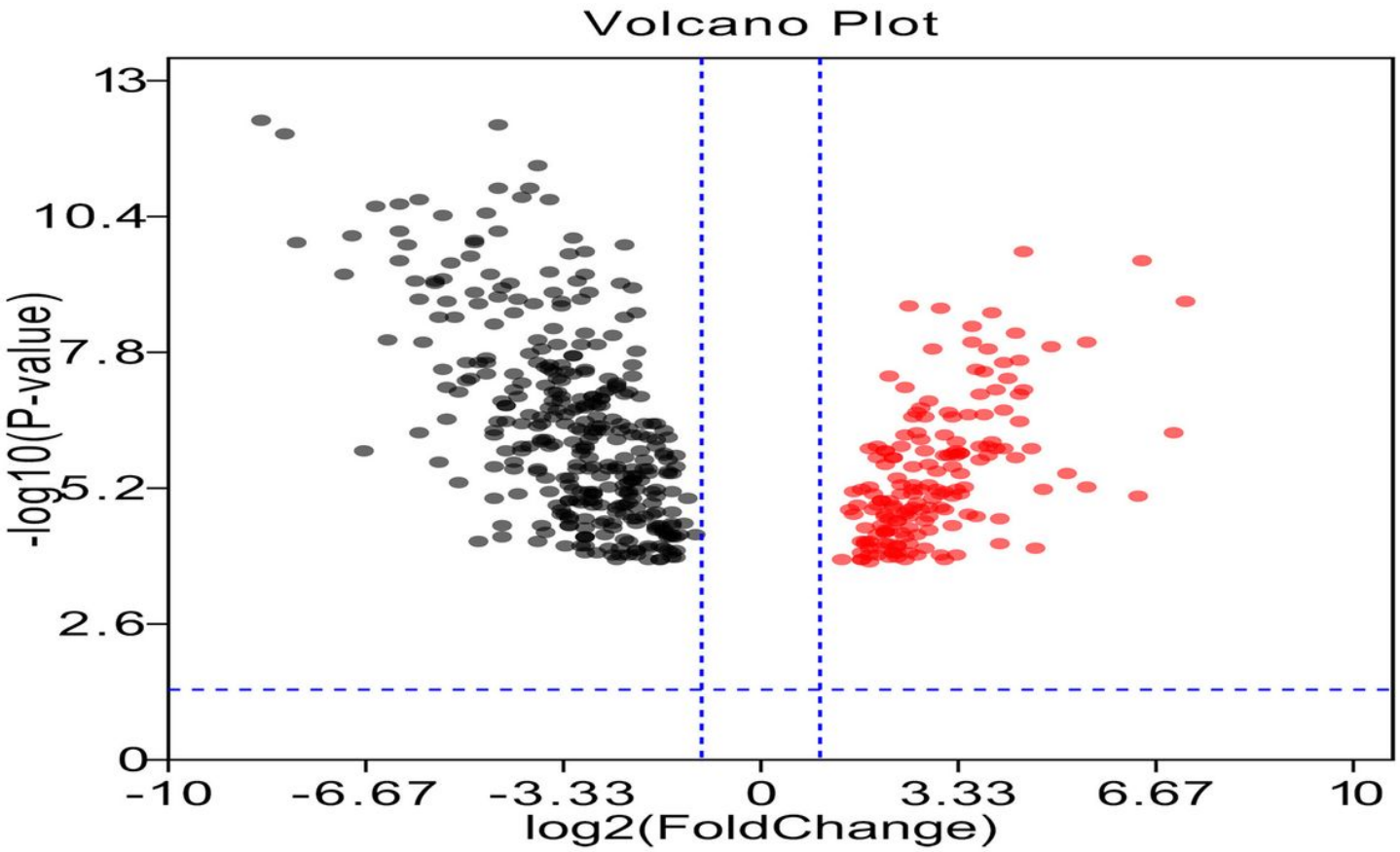

Figure 3

Volcano plot of the 514 identified DEGs. Red indicates 171 upregulated DEGs with a logFC>1. Black indicates 343 downregulated DEGs with a logFC<-1. DEG, differentially expressed gene; FC, fold change

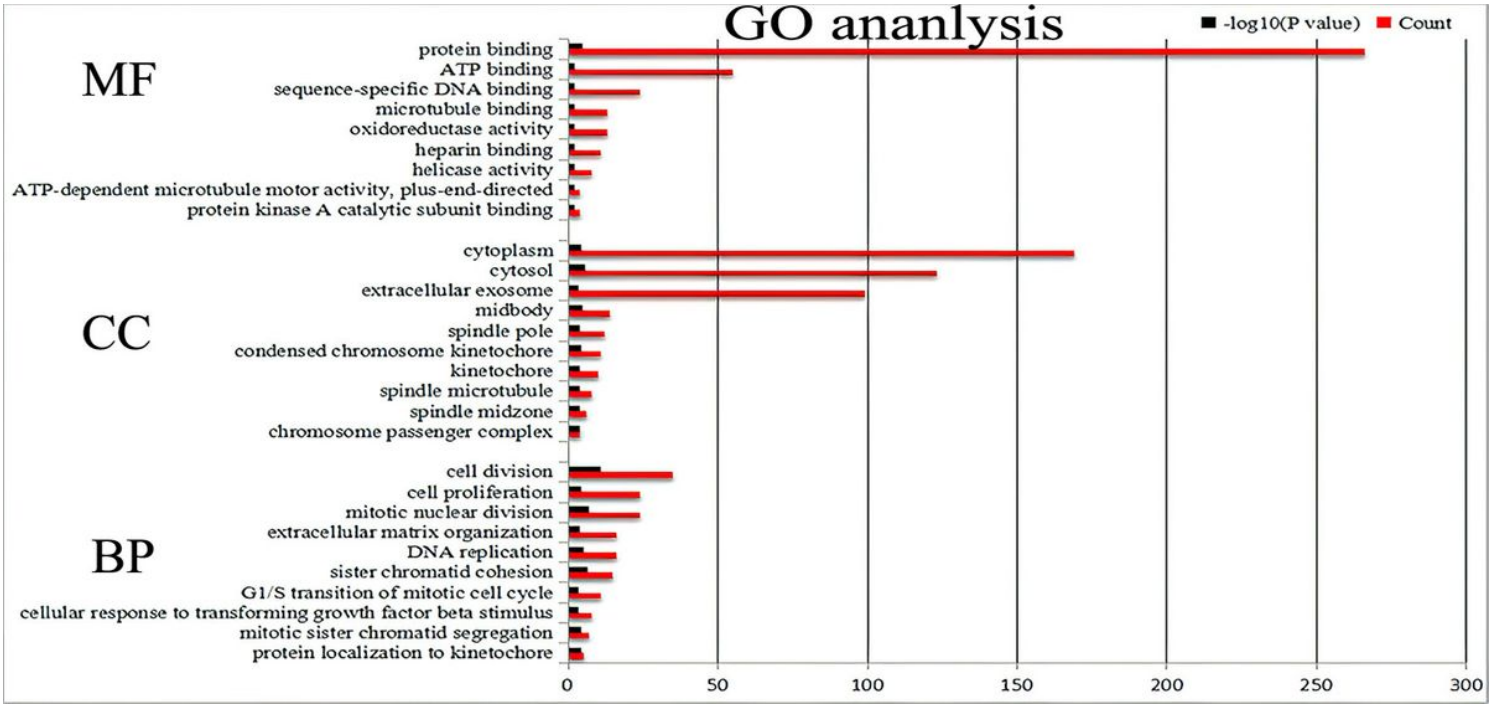

Figure 5

GO analysis of the DEGs; Red and black straight bars represent gene number and P-value, respectively. GO, gene ontology; DEG, differentially expressed gene; BP, biological process; CC, cell component; MF, molecular function

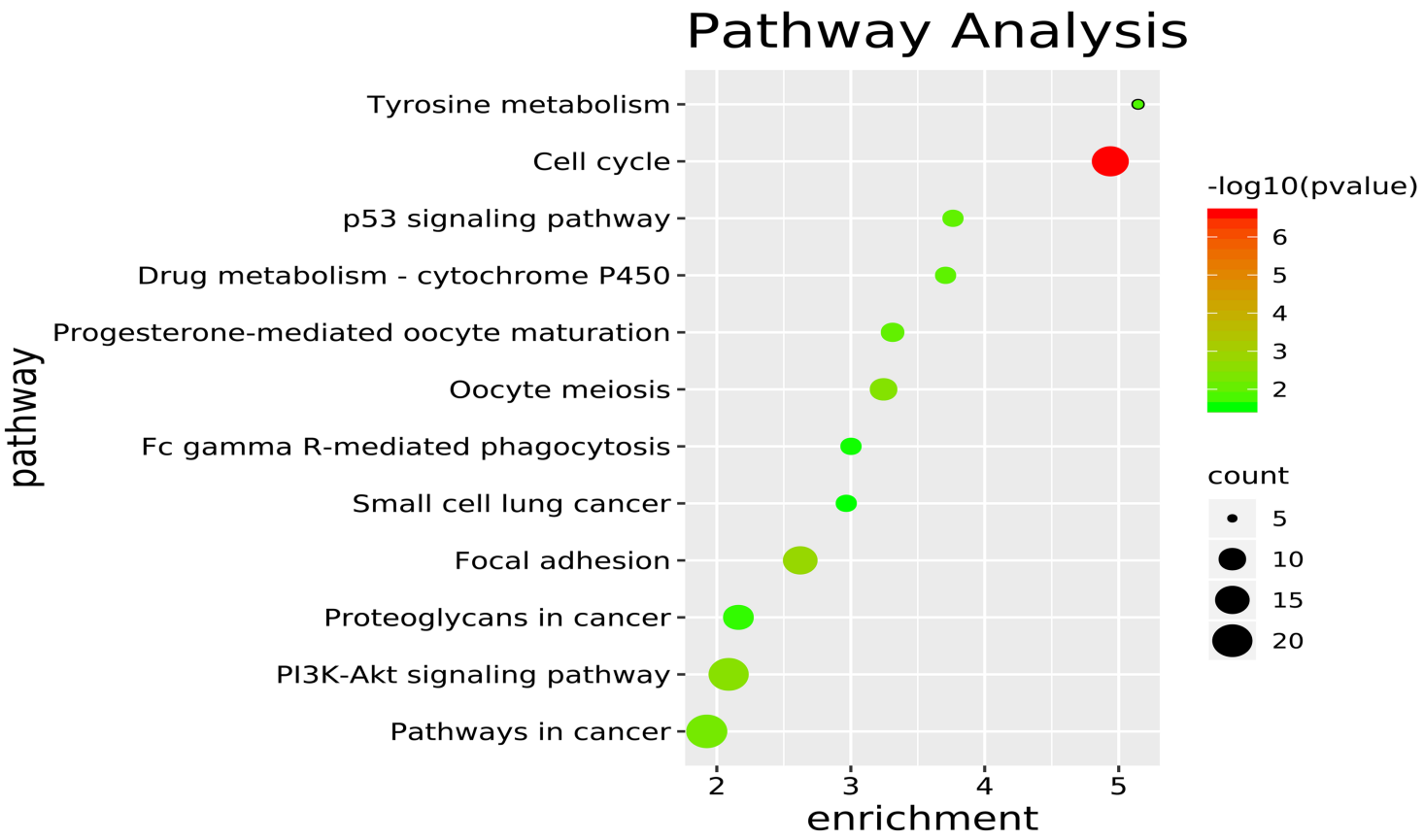

Figure 7

KEGG enrichment analysis of the 514 DEGs. The ordinate is the enrichment pathway name; abscissa is the enrichment of KEGG. Bubble size indicates the number of genes located in the functional area. Bubble color indicates the change of P-value. DEG, differentially expressed gene; KEGG, Kyoto Encyclopedia of Genes and Genomes

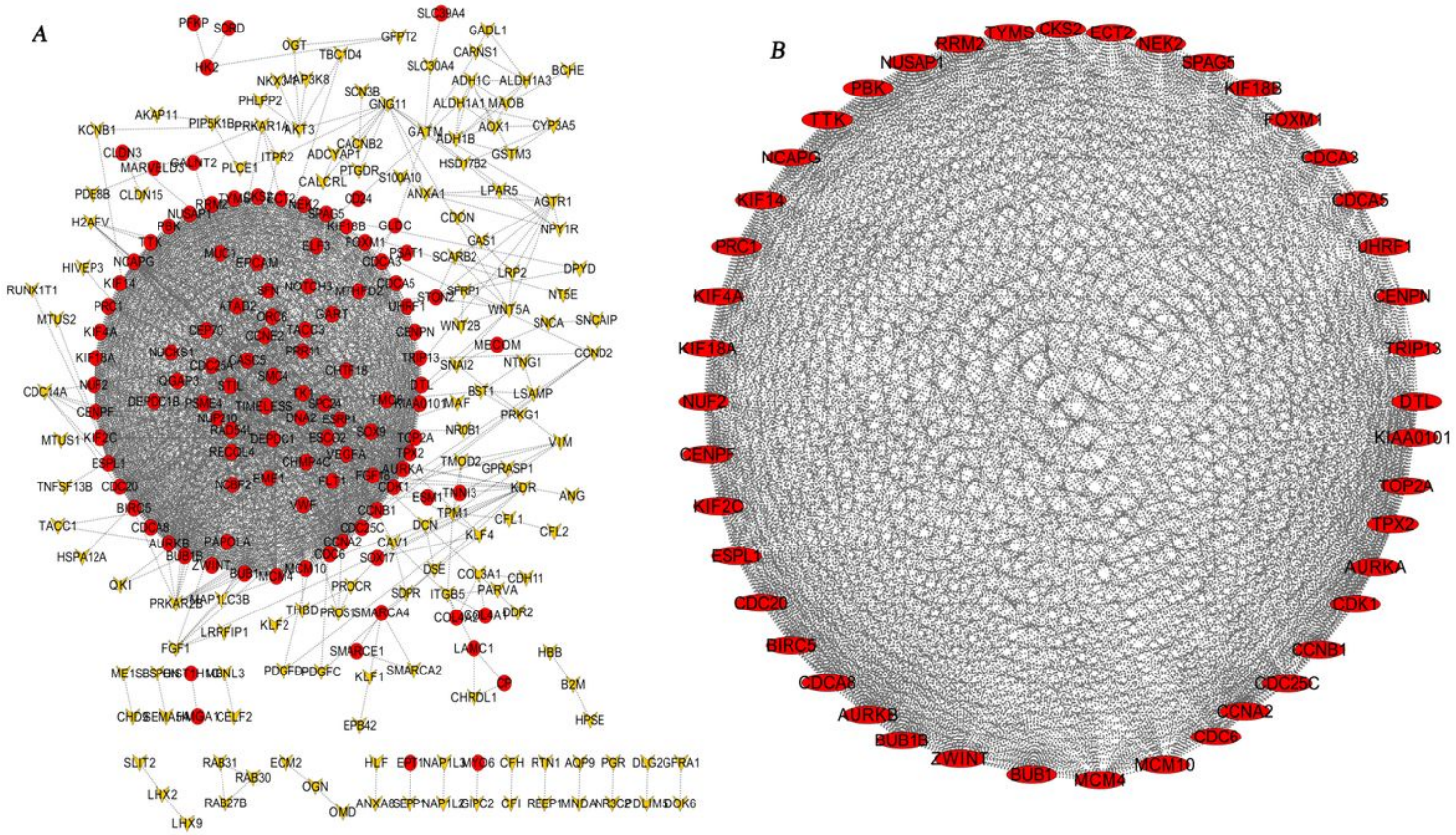

Figure 9

(a) Protein-protein network of 514 DEGs. (b) The most significant module was obtained from PPI network. upregulated genes are marked in red; down-regulated genes are marked in yellow; DEG, differentially expressed gene; PPI, protein-protein interaction

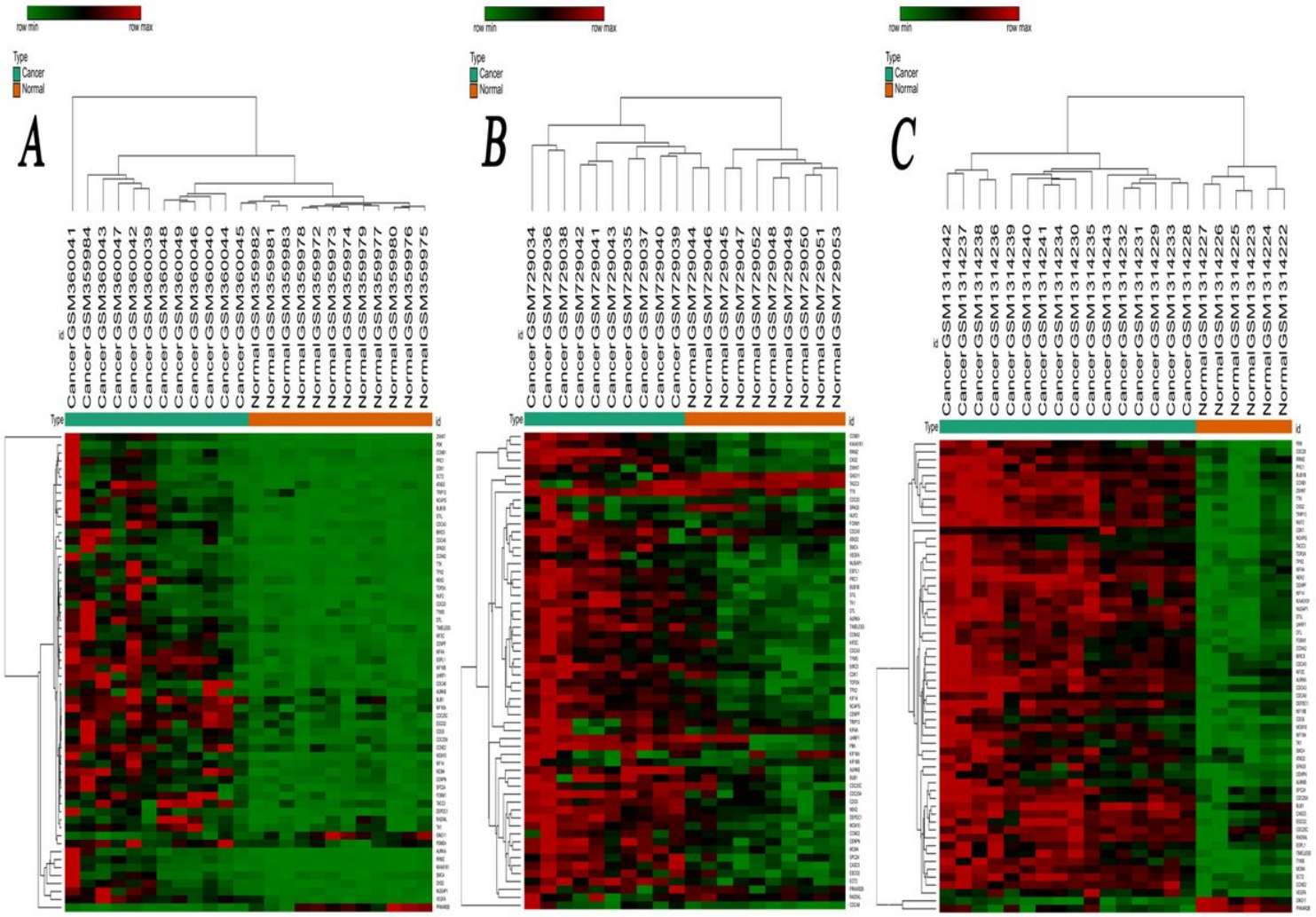

Figure 11

Heatmap of top 60 common DEGs in GSE14407 (a), GSE29450 (b) and GSE54388 (c) microarrays. The abscissa is the sample; ordinate is the gene name. Gene expression levels were indicated by colors as shown by the row, red represents high expression level and green represents low expression level.

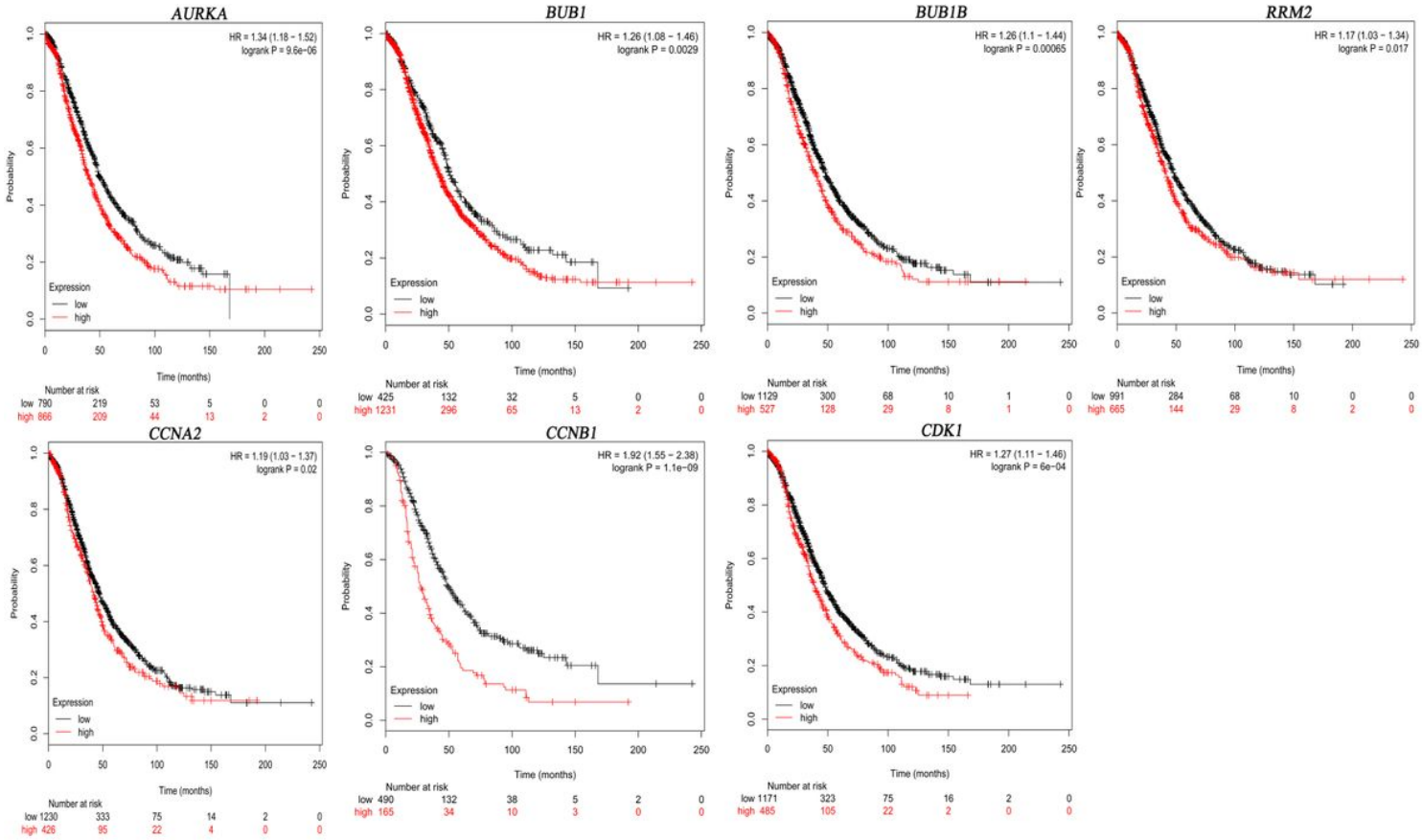

Figure 13

Overall survival analyses of seven hub genes (AURKA, BUB1B, CCNA2, CCNB1, CDK, RRM2, BUB1) were performed using Kaplan-Meier plotter online tools ($P < 0.05$)

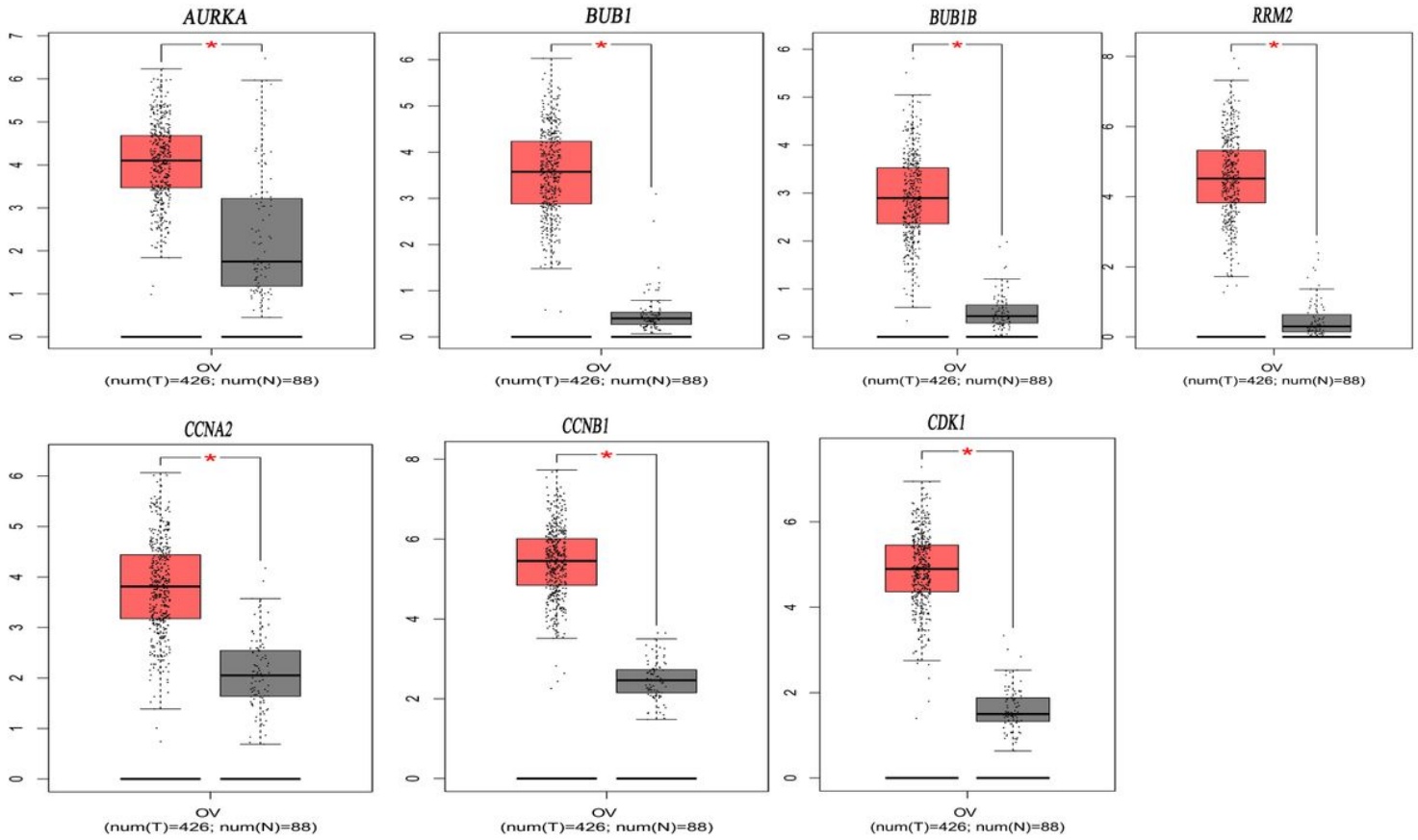

Figure 15

Analyzing the difference in hub gene expression between normal and cancer tissues through the GEPIA web server. Red color presents tumor tissues and grey color means normal tissues

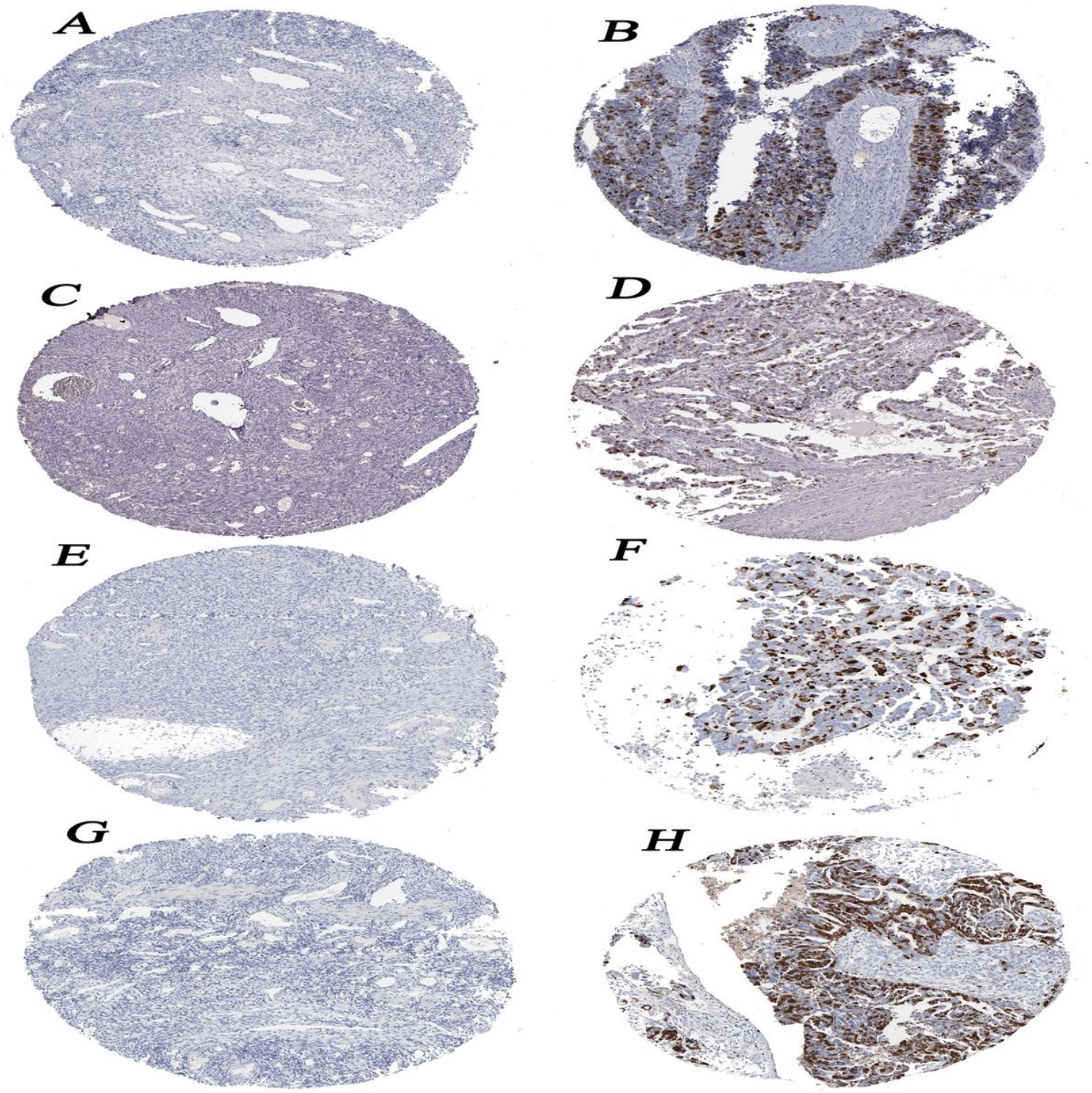

Figure 17

Validation of the hub genes from the HPA database. a: AURKA expression was Not detected in the normal samples (antibody CAB001454). b: Ovarian cancer samples displayed high expression levels of AURKA (antibody CAB001454). c: CCNA2 expression was not detected in the normal samples (antibody CAB000114). d: Ovarian cancer samples displayed intermediate expression levels of CCNA2 (antibody CAB000114). e: CCNB1 expression was not detected in the normal samples (antibody CAB003804). f: Ovarian cancer samples displayed high expression levels of CCNB1 (antibody CAB003804). g: CDK1

expression was not detected in the normal samples (antibody CAB003799). h: Ovarian cancer samples displayed high expression levels of CDK1 (antibody CAB003799).

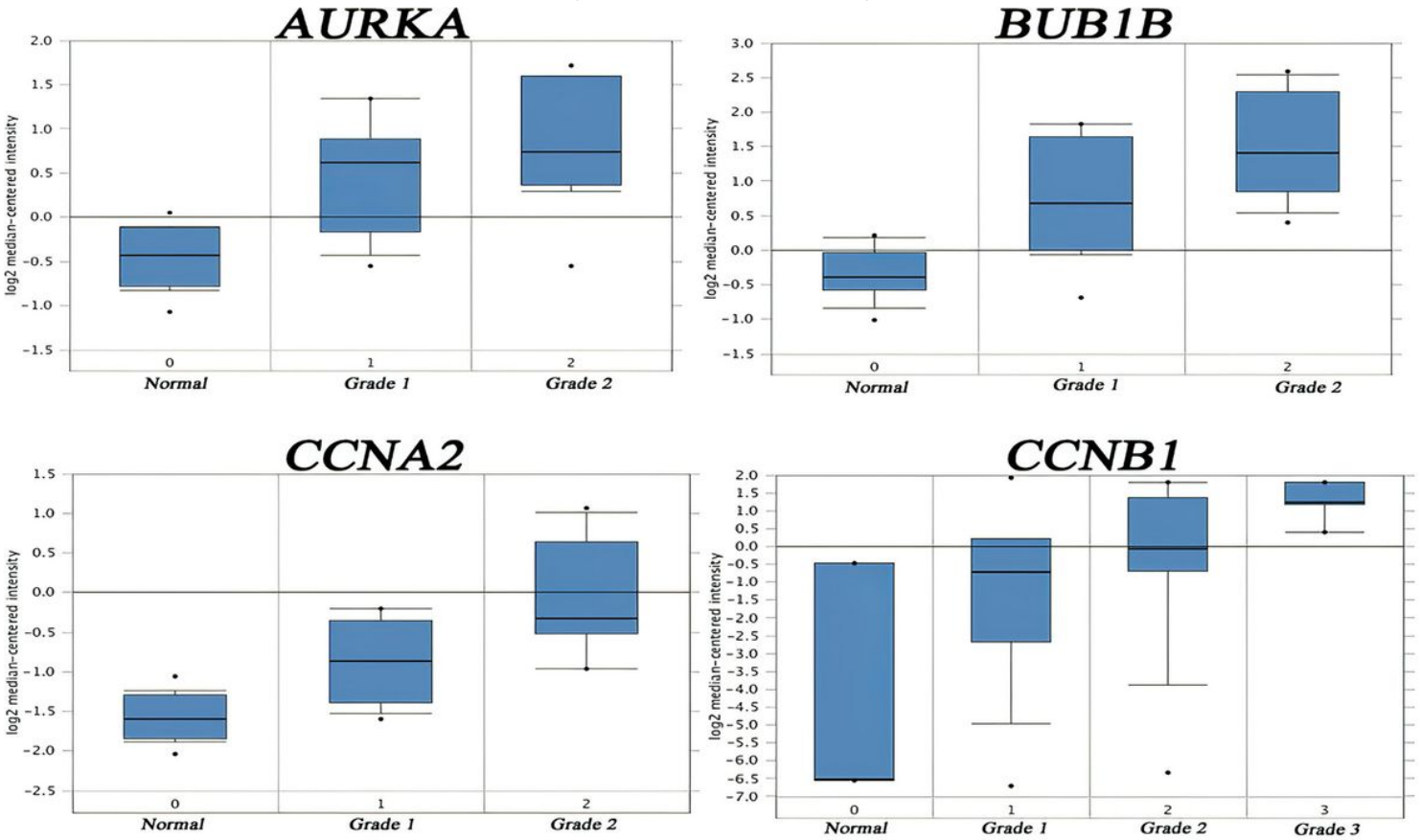

Figure 19

The association between the expression patterns of AURKA, BUB1B, CCNA2, CCNB1 and tumor grades through oncomine analysis online tool.

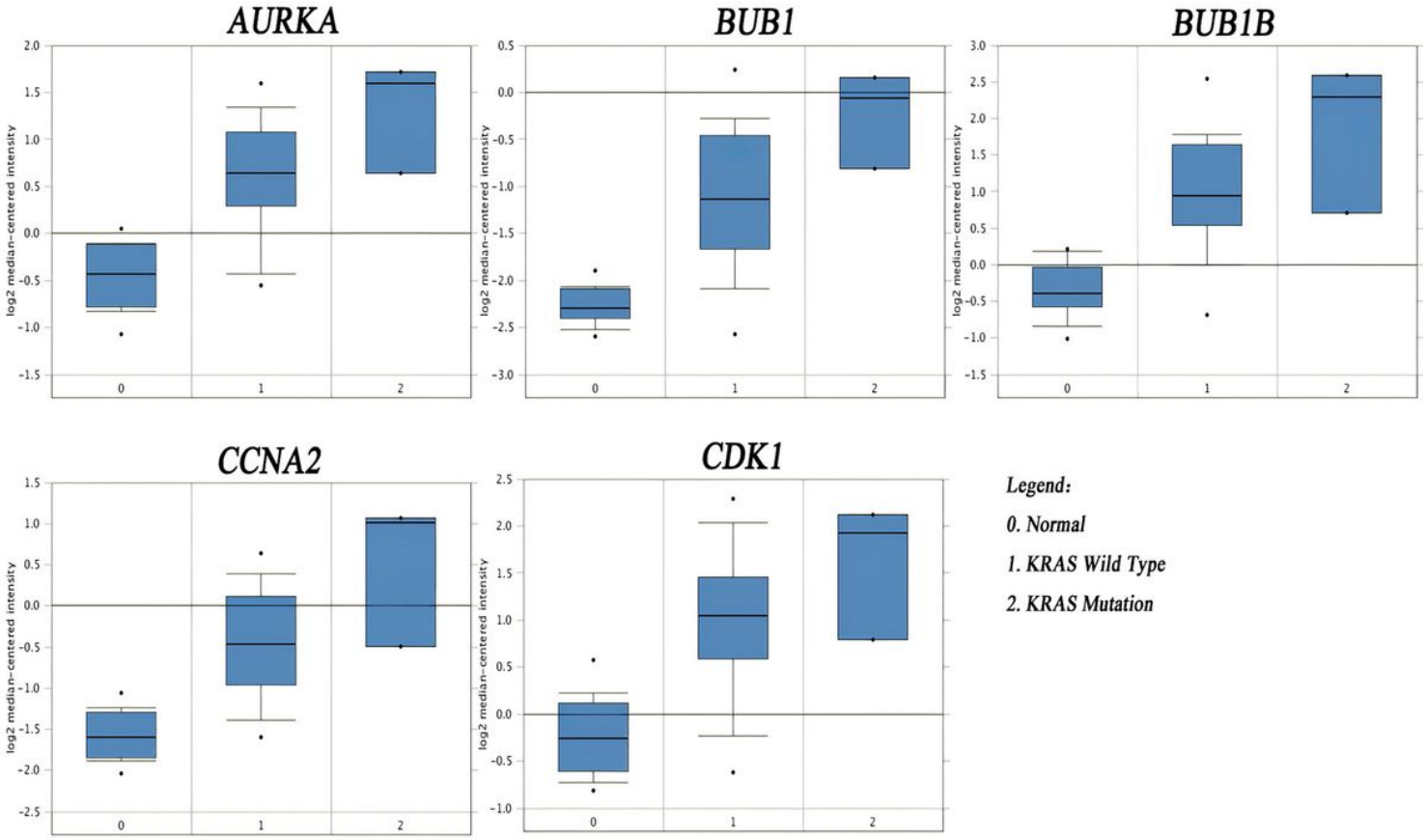

Figure 21

The association between the expression patterns of AURKA BUB1 BUB1B CDK1 CCNA2 and KRAS mutation status through oncomine analysis online tool.

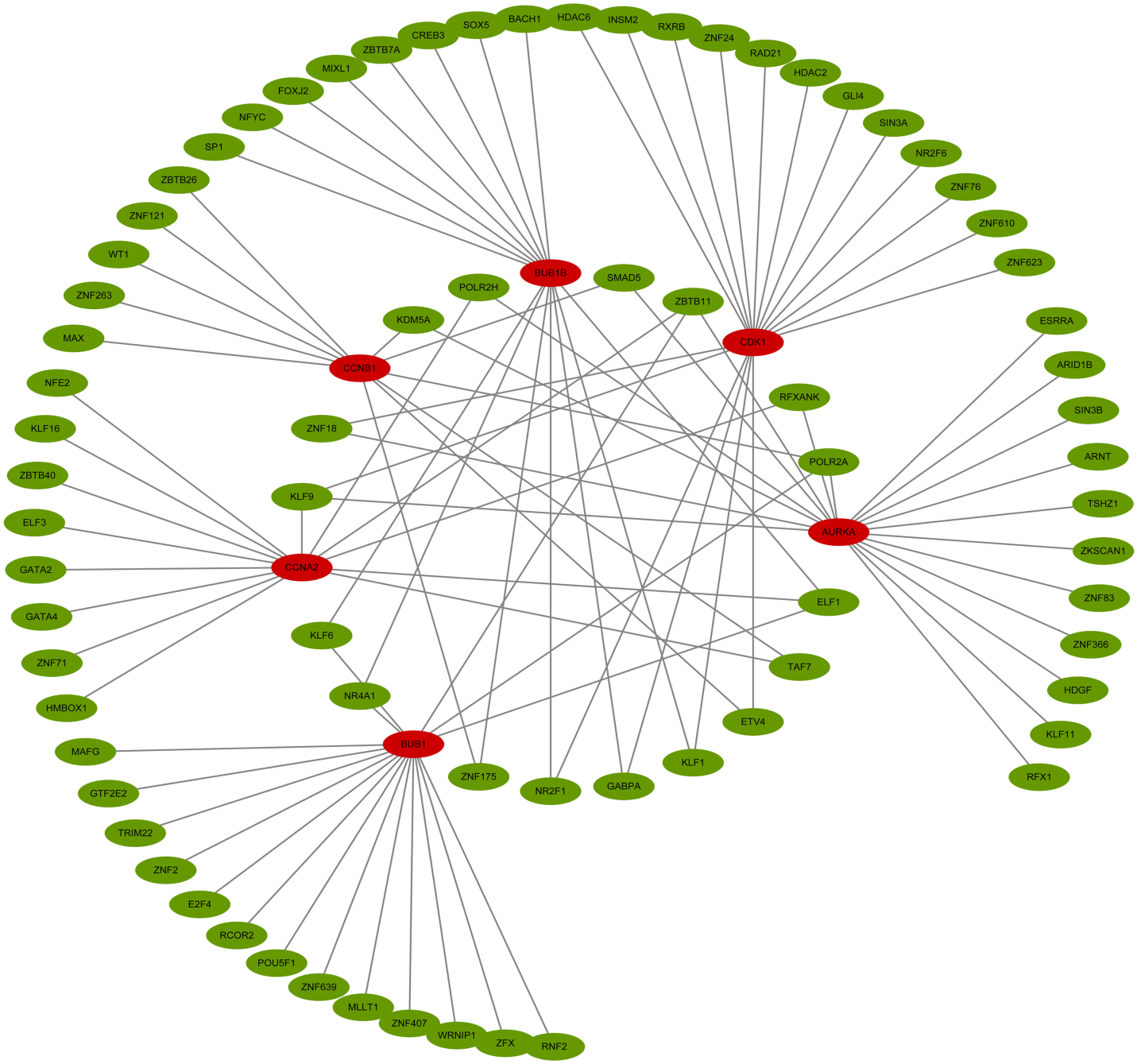

Figure 23

The TFs regulatory network and association with the hub genes. The red and green nodes represent genes and transcriptional factors, respectively. TFs, transcriptional factors.