Identification of significant genes with poor prognosis in ovarian cancer via bioinformatical analysis

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Abstract

Ovarian cancer (OC) is the highest frequent malignant gynecologic tumor with very complicated pathogenesis. The purpose of the present academic work was to identify significant genes with poor outcome and their underlying mechanisms. Gene expression profiles of GSE36668, GSE14407 and GSE18520 were available from GEO database. There are 69 OC tissues and 26 normal tissues in the three profile datasets. Differentially expressed genes (DEGs) between OC tissues and normal ovarian (OV) tissues were picked out by GEO2R tool and Venn diagram software. Next, we made use of the Database for Annotation, Visualization and Integrated Discovery (DAVID) to analyze Kyoto Encyclopedia of Gene and Genome (KEGG) pathway and gene ontology (GO). Then protein-protein interaction (PPI) of these DEGs was visualized by Cytoscape with Search Tool for the Retrieval of Interacting Genes (STRING). There were total of 216 consistently expressed genes in the three datasets, including 110 up-regulated genes enriched in cell division, sister chromatid cohesion, mitotic nuclear division, regulation of cell cycle, protein localization to kinetochore, cell proliferation and Cell cycle, progesterone-mediated oocyte maturation and p53 signaling pathway, while 106 down-regulated genes enriched in palate development, blood coagulation, positive regulation of transcription from RNA polymerase II promoter, axonogenesis, receptor internalization, negative regulation of transcription from RNA polymerase II promoter and no significant signaling pathways. Of PPI network analyzed by Molecular Complex Detection (MCODE) plug-in, all 33 up-regulated genes were selected. Furthermore, for the analysis of overall survival among those genes, Kaplan–Meier analysis was implemented and 20 of 33 genes had a significantly worse prognosis. For validation in Gene Expression Profiling Interactive Analysis (GEPIA), 15 of 20 genes were discovered highly expressed in OC tissues compared to normal OV tissues. Furthermore, four genes (BUB1B, BUB1, TTK and CCNB1) were found to significantly enrich in the cell cycle pathway via re-analysis of DAVID. In conclusion, we have identified four significant up-regulated DEGs with poor prognosis in OC on the basis of integrated bioinformatical methods, which could be potential therapeutic targets for OC patients.

Keywords: Bioinformatical analysis, Microarray, Ovarian cancer, Differentially expressed gene

Background

Ovarian cancer (OC) is the fifth cause of cancerous death among women all over the world [1]. Although some prognostic biomarkers have been exploited, the overall survival of OC remains weak due to its difficulty in early detection, distant metastasis and rapid dissemination [2, 3]. Therefore, more reliable prognostic biomarkers should be explored as a target for improving the treatment effect and better understanding the underlying mechanism.

Gene chip which was used for more than ten years can quickly detect differentially expressed genes and was proved to be a reliable technique [4] that could make many slice data be produced and stored in public databases. Therefore, a large number of valuable clues could be explored for new research on the base of these data. Furthermore, many bioinformatical studies on OC have been produced in recent years [5], which proved that the
integrated bioinformatical methods could help us to further study and better exploring the underlying mechanisms.

In this study, first, we chose GSE36668, GSE18520 and GSE14407 from Gene Expression Omnibus (GEO). Second, we applied for GEO2R online tool and Venn diagram software to obtain the commonly differentially expressed genes (DEGs) in the three datasets above. Third, the Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to analyze these DEGs including molecular function (MF), cellular component (CC), biological process (BP) and Kyoto Encyclopedia of Gene and Genome (KEGG) pathways. Fourth, we established protein-protein interaction (PPI) network and then applied Cytotype MCODE (Molecular Complex Detection) for additional analysis of the DEGs which would identify some core genes. Moreover, these core DEGs were imported into the Kaplan Meier plotter online database for the significant prognostic information ($P < 0.05$). In addition, we furtherly validated the DEGs expression between OV cancer tissues and normal OV tissues via Gene Expression Profiling Interactive Analysis (GEPIA) ($P < 0.05$). Taken above, only 15 DEGs were qualified. Then, we re-analyzed these 15 DEGs for KEGG pathway enrichment. Finally, four DEGs (BUB1B, BUB1, TTK and CCNB1) were generated and significantly enriched in the cell cycle pathway especially in G2/M phase. In conclusion, the bioinformatic study of our study provides some additional useful biomarkers which could be an effective target for OC patients.

**Methods**

**Microarray data information**

NCBI-GEO is regarded as a free public database of microarray/gene profile and we obtained the gene expression profile of GSE36668, GSE18520 and GSE 14407 in ovarian cancer and normal ovarian tissues. Microarray data of GSE36668, GSE18520 and GSE14407 were all on account of GPL570 Platforms ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array) which included 4 OC tissues and 4 normal OV tissues, 53 OC tissues and 10 normal OV tissues and 12 OC tissues and 12 normal OV tissues, respectively.

**Data processing of DEGs**

DEGs between OC specimen and normal OV specimen were identified via GEO2R online tools [6] with $|\log FC| > 2$ and adjust $P$ value $< 0.05$. Then, the raw data in TXT format were checked in Venn software online to detect the commonly DEGs among the three datasets. The DEGs with log FC < 0 was considered as down-regulated genes, while the DEGs with log FC > 0 was considered as an up-regulated gene.

**Gene ontology and pathway enrichment analysis**

Gene ontology analysis (GO) is a commonly used approach for defining genes and its RNA or protein product to identify unique biological properties of high-throughput transcriptome or genome data [7]. KEGG is a collection of databases dealing with genomes, diseases, biological pathways, drugs, and chemical materials [8]. DAVID which is an online bioinformatic tool is designed to identify a large number of genes or proteins function [9]. We could use DAVID to visualize the DEGs enrichment of BP, MF, CC and pathways ($P < 0.05$).

**PPI network and module analysis**

PPI information can be evaluated by an online tool, STRING (Search Tool for the Retrieval of Interacting Genes) [10]. Then, the STRING app in Cytoscape [11] was applied to examine the potential correlation between these DEGs (maximum number of interactors = 0 and confidence score $\geq 0.4$). In addition, the MCODE app in Cytoscape was used to check modules of the PPI network (degree cutoff = 2, max. Depth = 100, k-core = 2, and node score cutoff = 0.2).

**Survival analysis and RNA sequencing expression of core genes**

Kaplan Meier-plotter are a commonly used website tool for assessing the effect of a great number of genes on survival based on EGA, TCGA database and GEO (Affymetrix microarrays only) [12]. The log rank $P$ value and hazard ratio (HR) with 95% confidence intervals were computed and showed on the plot. To validate these DEGs, we applied the GEPIA website to analyze the data of RNA sequencing expression on the basis of thousands of samples from the GTEx projects and TCGA [13].

**Results**

**Identification of DEGs in ovarian cancers**

There were 69 OC tissues and 26 normal OV tissues in our present study. Via GEO2R online tools, we extracted 1516, 1150 and 1670 DEGs from GSE36668, GSE18520 and GSE 14407, respectively. Then, we used Venn diagram software to identify the commonly DEGs in the three datasets. Results showed that a total of 216 commonly DEGs were detected, including 106 down-regulated genes ($\log FC < 0$) and 110 up-regulated genes ($\log FC > 0$) in the OC tissues (Table 1 & Fig. 1).

**DEGs gene ontology and KEGG pathway analysis in ovarian cancers**

All 216 DEGs were analyzed by DAVID software and the results of GO analysis indicated that 1) for biological processes (BP), up-regulated DEGs were particularly enriched in regulation of cell cycle, cell division, mitotic nuclear division, protein localization to kinetochore,
sister chromatid cohesion and cell proliferation, and down-regulated DEGs in blood coagulation, positive regulation of transcription from RNA polymerase II promoter, palate development, negative regulation of transcription from RNA polymerase II promoter, axonogenesis and receptor internalization; 2) for molecular function (MF), up-regulated DEGs were enriched in protein binding, ATP-dependent microtubule motor activity, protein kinase binding, plus-end-directed, microtubule binding, sequence-specific DNA binding and down-regulated DEGs in RNA polymerase II core promoter proximal region sequence-specific binding, RNA polymerase II transcription factor binding, RNA polymerase II core promoter proximal region sequence-specific binding and growth factor activity, transcriptional repressor activity, transcriptional activator activity; 3) for GO cell component (CC), up-regulated DEGs were significantly enriched in the nucleoplasm, midbody, spindle microtubule, spindle, cytosol and nucleus, and down-regulated DEGs in proteinaceous extracellular matrix anchored component of membrane, extracellular region and extracellular space (Table 2).

KEGG analysis results were shown in Table 3 which demonstrated that up-regulated DEGs were particularly enriched in p53 signaling pathway, cell cycle and progesterone-mediated oocyte maturation while down-regulated DEGs in no significant signaling pathways ($P < 0.05$).

Table 1 All 216 commonly differentially expressed genes (DEGs) were detected from three profile datasets, including 106 down-regulated genes and 110 up-regulated genes in the OC tissues compared to normal OV tissues

| DEGs          | Genes Name                                                                 |
|---------------|-----------------------------------------------------------------------------|
| Up-regulated  | C1orf106 MPZL2 EHF KLK6 MMP7 KLHL14 IGF2BP3 CCNB1 FOXQ1 PROM2 SUSD2 CLDN4 DEF81 MEOX1 SMIM22 KLK8 FOXM1 CDK1 SORT1 MUC1 KIF11 ELF3 E2F1 FOLR1 MAL SULT1C2 CENPU STON2 GRHL2 KIF14 KCCAT333 AURKB MTHFD2 LOC101929219 LOC100565620 C1orf186 RAI1A1217 KIF4A MCM10 EBS SOX17 EPHX4 CDH4 MEUK CDCL2 OXCG5 AIF1 LCDC2 INHBB BUB1 PRR11 TRIP13 CDA5 SLC2A1 DUXAP10 EPAM HMGA2 RGS1 ECT2 DEPC1 MITF2 PARM3 UBE2C CCNB2 LOC100288637 ARHGAP11B CRABP2 CD24 LINC00673 LINCO00511 PRS52 LOC613266 TCC39A PRC1 P53T1 LRP8 PTHFR2 SC78 CDY1B1 CTNNB3 HMGR C12orf57 GPM6B LOC101928554 CENPK LCN2 PRAME RAI1A0101 HMGA1 TTK NCA V ServiceProvider=LOC1012966 DUXAP10 NEK2 CENPF NUSAP1 S6GALNA1 |
| Down-regulated| MUM1L1 NAP1L2 CYP2U1 VGLL3 GHR NEFH TMEM255A PPM1K TSPAN8 BAMBI MCIU3 O101930363 LOC101928349 LOC100507387 FAM153C FAM153A FAM153B LOC100507387 FAM153A FAM153B BDH2 DPDY ANTXR2 HLF PRSS35 THBD PRRX1 L7Y5-CDD02 L7Y5-CDD02 L7Y5-ABCAB WDR17 ZFPM2 OMD TCF21 PDGF DLF2 SNAIP1 NEGR1 NTSE RUNX1 T1 TRPC1 SNCA PLEKH2 GAS1RR MTUS1 GPM6A CPE1 MGARP LSAMP EEFM1 P3GALT2 CHGB DIRAS3 PRKAR2B FAM13C KNT2 TEM150C ECM2 GIPC2 OGN SNX29P2 ARX TCEAL2 NAP1L3 SDPR TCEAL7 NBEA Cxorf57 CSGALNAC1 CYS1 CNTN1 AKAP12 ME03X COL4A1 CALCR ALDH1A1 SMPD3 TXB3 WNT2B ANKRD29 NR2F1-AS1 MCC CBNL4 CELF2 ITM2A GNG11 PGR OGFOD1 TFP1 GRASP1 PEG3 PCDH9 HAND2-AS1 RMR35 FGF13 PRDM5 MFEP8 RIGL1 TLE4 DCEX PXSX BCN2 GATM RNF128 LHX9 AOX1 AKT3 OLFML1 RNA54 GATA4 NXRPH2 NDN LOC100506718 FFLR2 |

Fig. 1 Authentication of 216 common DEGs in the three datasets (GSE36668, GSE18520 and GSE14407) through Venn diagrams software (available online: http://bioinformatics.psb.ugent.be/webtools/Venn/). Different color meant different datasets. a. 110 DEGs were up-regulated in the three datasets (logFC > 0). b. 106 DEGs were down-regulated in three datasets (logFC < 0).
Protein–protein interaction network (PPI) and modular analysis

A total of 107 DEGs were imported into the DEGs PPI network complex which included 107 nodes and 698 edges, including 60 down-regulated and 47 up-regulated genes (Fig. 2a). There were total 109 of the 216 DEGs which were not contained into the DEGs PPI network (Fig. 2a). Then we applied Cytotype MCODE for further analysis and results showed that 33 central nodes which were all up-regulated genes were identified among the 107 nodes (Fig. 2b).

Analysis of core genes by the Kaplan Meier plotter and GEPIA
Kaplan Meier plotter (http://kmplot.com/analysis) was utilized to identify 33 core genes survival data. It was found that 20 genes had a significantly worse survival
while 13 had no significant (P < 0.05, Table 4 & Fig. 3). Then, GEPIA was used to dig up the 20 gene expression level between cancerous and normal people. Results reported that 15 of 20 genes reflected high expressed in OC samples contrasted to normal OV samples (P < 0.05, Table 5 & Fig. 4).

Re-analysis of 15 selected genes via KEGG pathway enrichment
To understand the possible pathway of these 15 selected DEGs, KEGG pathway enrichment was re-analyzed via DAVID (P < 0.05). Results showed that four genes (BUB1B, BUB1, TTK and CCNB1) markedly enriched in the cell cycle pathway (P = 1.1E-4, Table 6 & Fig. 5).

Discussion
To identify more useful prognostic biomarkers in OV cancer, this study used bioinformatical methods on the basis of three profile datasets (GSE36668, GSE18520 and GSE 14407). Sixty-nine ovarian cancer specimens and twenty-six normal specimens were enrolled in the present research. Via GEO2R and Venn software, we revealed a total of 216 commonly changed DEGs (|logFC| > 2 and adjust P value < 0.05) including 110 up-regulated (Log FC > 0) and 106 down-regulated DEGs (Log FC < 0). Then, Gene Ontology and Pathway Enrichment Analysis using DAVID methods showed that 1) for biological processes (BP), up-regulated DEGs were particularly enriched in regulation of cell cycle, cell division, mitotic nuclear division, protein localization to kinetochore, sister chromatid cohesion and cell proliferation, and down-regulated DEGs in blood coagulation, positive regulation of transcription from RNA polymerase II promoter, palate development, negative regulation of transcription from RNA polymerase II promoter, axonogenesis and receptor internalization; 2) for molecular function (MF), up-regulated DEGs were enriched in ATP-dependent microtubule motor activity, protein binding, plus-end-directed, microtubule binding, sequence-specific DNA binding, protein kinase binding and down-regulated DEGs in transcriptional repressor activity, RNA polymerase II core promoter proximal region

| Pathway ID   | Name                           | Count | %     | p-Value    | Genes                                      |
|--------------|--------------------------------|-------|-------|------------|--------------------------------------------|
| hsa04110     | Cell cycle                     | 8     | 7.62  | 7.31E-07   | CCNB1, E2F1, CDK1, CCNB2, BUB1, TTK, BUB1B, CDC20 |
| hsa04115     | p53 signaling pathway          | 4     | 3.81  | 0.002934   | CCNB1, CDK1, CCNB2, RRM2                    |
| hsa04914     | Progesterone-mediated oocyte maturation | 4     | 3.81  | 0.006123   | CCNB1, CDK1, CCNB2, BUB1                    |
sequence-specific binding and growth factor activity, RNA polymerase II core promoter proximal region sequence-specific binding, RNA polymerase II transcription factor binding, transcriptional activator activity; 3) for GO cell component (CC), up-regulated DEGs were significantly enriched in the nucleoplasm, midbody, spindle microtubule, spindle, cytosol and nucleus, and down-regulated DEGs in proteinaceous extracellular matrix anchored component of membrane, extracellular space and extracellular region. For pathway analysis, up-regulated DEGs were particularly enriched in p53 signaling pathway, cell cycle and progesterone-mediated oocyte maturation and while down-regulated DEGs in no noteworthy signaling pathways \((P < 0.05)\). Next, DEGs PPI network complex of 108 nodes and 698 edges was constructed via the STRING online database and Cytoscape software. Then, 33 vital up-regulated genes were screened from the PPI network complex by Cytotype MCODE analysis. Furthermore, through Kaplan Meier plotter analysis, we found that 20 of 33 genes had a significantly worse survival. In validating these 20 genes, 15 genes reflected high expression in OC samples compared with normal samples by GEPIA analysis \((P < 0.05)\). Finally, we re-analyzed 15 genes via DAVID for KEGG pathway enrichment and found that four genes (BUB1B, BUB1, TTK and CCNB1) enriched in cell cycle had a significance \((P < 0.05)\) which could be considered as new effective targets to improve the prognosis of OC patients.

Mitotic checkpoint serine/threonine kinase B (BUB1B), which is seen as a mammalian homolog of yeast Mad3, but they are significantly different because BUB1B has a kinase domain which is not found in Mad3 [14]. In 2004, Kops GJ, et al. reported that apoptotic cell death and massive chromosome loss could occur due to the inhibition of BUB1B kinase activity and reduction of the BUB1B level in human cancer cells [15]. BUB1B has been demonstrated to enhance tumor proliferation and is associated with worse survival rate in several types of cancer, including prostate cancer, breast, gastric and colorectal [16–19]. Another study proved that knockdown of BUB1B resulted in inhibition of tumor growth in vivo, including the regression of established tumors via postmitotic endoreduplication checkpoint [20] which is the replication of the genome during the cell cycle.

### Table 4 The prognostic information of the 33 key candidate genes

| Category                | Genes                                                                 |
|-------------------------|------------------------------------------------------------------------|
| Genes with significantly worse survival \((P < 0.05)\) | BUB1 BUB1B CCNB1 CDCAS CENPF CENPK DEPDC1 ECT2 FAM83D FOXM1 HMMR KIF11 KIF14 KIF15 MCM10 NCAPG RAD51AP1 TIMELESS TTK UBE2C |
| Genes without significantly worse survival \((P > 0.05)\) | AURKB CCNB2 CDC20 DTL E2F1 KIAA0101 KIF4A MELK NEK2 NUSAP1 PRC1 RRM2 TRIP13 |

### Table 5 Validation of 20 genes via GEPIA

| Category                | Genes                                                                 |
|-------------------------|------------------------------------------------------------------------|
| Genes with high expressed in OC \((P < 0.05)\) | BUB1 BUB1B CCNB1 CDCAS CENPF DEPDC1 ECT2 FAM83D FOXM1 HMMR KIF11 NCAPG RAD51AP1 TTK UBE2C |
| Genes without high expressed in OC \((P > 0.05)\) | CENPK KIF14 KIF15 MCM10 TIMELESS |

Fig. 3 The prognostic information of the 33 core genes. Kaplan meier plotter online tools were used to identify the prognostic information of the 33 core genes and 20 of 33 genes had a significantly worse survival rate \((P < 0.05)\)
without the subsequent completion of mitosis and/or cytokinesis [21].

BUB1 which is a serine/threonine kinase and encoded by the BUB1 gene, binds centromeres during mitosis. It has been noted that over-expressed BUB1 is related to several cancers and their worse clinical prognosis. Wang et al. [22] presented that high expression of BUB1 was associated with poor disease-free survival of 203 patients with breast cancer. In addition, Zhao et al. [23] indicated that higher positive percentage of BUB1 protein meant a more advanced stage and a higher differentiation degree of endometrial carcinoma. Furthermore, Pinto et al. [24] demonstrated that over-expression of BUB1 was found to be substantially related to Furhman grade of the tumors and with the number of genomic copy number changes. By isolating daughter cells from mother cells, BUB1 were vitally responsible for the accurate assignment of chromosomes without establishing the mitotic spindle checkpoint and aligning chromosomes [25, 26].

Monopolar spindle1 (Mps1, also known as TTK), is a bispecific protein kinase that phosphorylates serines/threonines and tyrosines [27]. Mps1 is a core segment of the SAC (spindle assembly checkpoint) and is a key monitoring mechanism to ensure healthy cell proliferation and precise division [28, 29]. In addition to mitotic SAC regulation, Mps1 play roles in other processes, including DNA damage response, centrosome duplication and organ development [30]. Moreover, high expression of Mps1 was easily found in several human malignancies, such as thyroid carcinoma, glioblastoma and breast cancer [31–34].

CCNB1, G2/Mitotic-specific cyclin B1, is a monitoring protein in mitosis and expressed primarily in G2/M phase which is critical for controlling the cell cycle at the G2/M (mitosis) transition. Recently, increasing evidence demonstrated that CCNB1 was over-expressed in considerable cancers with poor prognosis, including gastric cancer [35], esophageal squamous cell carcinoma [36], non-small cell lung cancer [37] and astrocytomas [38]. Furthermore, it was also pointed out that down-regulation of CCNB1 of mRNA levels and protein could reduce cell proliferation [39]. In 2017, Zhao P, et al. reported that up-regulation of CCNB1 could be an index

![Fig. 4](image_url) Significantly expressed 20 genes in OV cancer patients compared to healthy people. To further identify the genes' expression level between OV cancer and normal people, 20 genes which were related with poor prognosis were analyzed by GEPIA website. 15 of 20 genes had significant expression level in OV cancer specimen compared to normal specimen ($P < 0.05$). Red color means tumor tissues and grey color means normal tissues.

| Pathway ID | Name                                | Count | %   | p-Value   | Genes      |
|------------|-------------------------------------|-------|-----|-----------|------------|
| hsa04110   | Cell cycle                          | 4     | 26.7| 1.1E-04   | CCNB1 BUB1 TTK BUB1B |
| hsa04914   | Progesterone-mediated oocyte maturation | 2     | 13.3| 7.3E-02   | CCNB1 BUB1  |

Table 6 Re-analysis of 15 selected genes via KEGG pathway enrichment.
for pituitary adenomas invasiveness and played a part in the pathology of pituitary adenomas with other monitoring molecules in the cell cycle [40].

Numerous studies have proved that these four genes were related to various types of cancer’s progression, however, very few studies have been reported about these four genes in OV cancer after we searched these four genes in Pubmed website. Therefore, the data in our study could provide useful information and direction for future study in OV cancer.

Conclusions

Taken above, our bioinformatics analysis study identified four DEGs (BUB1B, BUB1, TTK and CCNB1) between OC tissues and normal OV tissues on the base of three different microarray datasets. Results showed that these four genes could play key roles in the progression of OC. However, these predictions should be verified by a series of experiments in the future. Anyway, these data may provide some useful information and direction into the potential bio-markers and biological mechanisms of OC.

Abbreviations

BP: Biological process; BUB1B: Mitotic checkpoint serine/threonine kinase B; CC: Cellular component; CCNB1: G2/Mitotic-specific cyclin B1; DAVID: Database for Annotation, Visualization and Integrated Discovery; DEGs: Differentially expressed genes; GEO: Gene Expression Omnibus; GEPI A: Gene Expression Profiling Interactive Analysis; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Gene and Genome; MCODE: Molecular Complex Detection; MF: Molecular function; OC: Ovarian cancer; OV: Ovarian; PPI: protein-protein interaction; STRING: Search Tool for the Retrieval of Interacting Genes

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Availability of data and materials

The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Authors’ contributions

XY Y and JJ Z conceived and designed the idea to this paper; HF and ZY G collected and analyzed the data, and drafted the paper; QL and QH L analyzed the data and revised the final paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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