Original Article



# Identification of critical genes in gastric cancer to predict prognosis using bioinformatics analysis methods

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**Background:** Ranking fourth in the world in tumor incidence and second in cancer-related death worldwide, gastric cancer (GC) is one of the major malignant tumors, and has a very complicated pathogenesis. In the present study, we aimed to identify new biomarkers to predict the survival rate of GC patients.

**Methods:** The differentially expressed genes (DEGs) between GC tissues and normal stomach tissues were obtained by using GEO2R, and overlapped DEGs were acquired with Venn diagrams. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were conducted with R software. Then, the protein-protein interaction (PPI) of these DEGs was visualized by Cytoscape. Gene Expression Profiling Interactive Analysis (GEPIA) was used to verify the expression differences of hub genes in gastric adenocarcinoma tissues and normal tissues. Overall survival (OS) of hub genes was calculated by Kaplan-Meier plotter.

**Results:** There were a total of 128 consistently expressed genes in the two datasets: 85 upregulated genes were enriched in extra-cellular matrix (ECM)-receptor interaction, protein digestion and absorption, focal adhesion, gastric acid secretion, mineral absorption, systemic lupus erythematosus, amoebiasis, and PI3K-Akt signaling pathway, and 43 downregulated genes were 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 in no significant signaling pathways. From the PPI network analyzed by Molecular Complex Detection (MCODE) plug-in, all 27 upregulated genes were selected. Furthermore, to analyze the OS among these genes, Kaplan–Meier analysis was conducted, and 25 genes were associated with remarkably worse survival. For validation in GEPIA, 11 of 25 genes were discovered to be highly expressed in GC tissues compared to normal OS tissues. Furthermore, in the re-analysis of the Database for Annotation, Visualization and Integrated Discovery (DAVID), three genes [G2/miotic-specific cyclin B1 (CCNB1), polo-like kinases 1 (PLK1), and pituitary tumor-transforming gene-1 (PTTG1)] were markedly enriched in the cell cycle pathway, particulary the G1-G1/S phase.

**Conclusions:** Three remarkably upregulated DEGs with poor prognosis in GC were identified and may serve as new prognostic biomarkers and targets in GC therapy.

**Keywords:** Bioinformatical analysis; gastric cancer (GC); survival; differentially expressed genes (DEGs); G2/miotic-specific cyclin B1 (CCNB1); polo-like kinases 1 (PLK1); pituitary tumor-transforming gene-1 (PTTG1)

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## Introduction

As one of the most heterogeneous, multifactorial diseases in the world, gastric cancer (GC) persists in its high morbidity and mortality, especially in some areas of China (1). It is damaging to human physical and mental health, and thus also aggravates the public health and economic burden of China (2,3). Even though the swift development of technologies like gastroscopy and computed tomography has enabled early diagnosis and treatment to greatly slow the deleterious progression of GC, some patients still suffer from unusual patterns of locoregional and systemic recurrence. Furthermore, a large portion of patients still progress to the advanced stage of GC for various reasons. For these reasons, the overall survival (OS) of GC patients remains low around the world. Indeed, GC, as the fourth most common global malignancy, also ranks as the world's second leading cause of cancer-related death (4,5). Alarmingly, the incidence of GC is gradually increasing in young people (6). Even though some prognostic biomarkers have been identified and applied in clinical treatment (7), there is a still an urgent need to search for other significant genes in GC in order to better understand the underlying mechanism and improve the treatment effect. Gene chip has been used for several decades and has proven to be a reliable technique. Using it, differentially expressed genes (DEGs) can be detected quickly, and gene chip may even be used to produce many genomic information for storage in public databases. This wealth of data could then be exploited as a base for a large number of investigative studies. Indeed, an increasing amount of bioinformatical studies on GC have been conducted, which gives assurance that the underlying mechanisms of GC can be explored with integrated bioinformatical methods.

In this study, we first selected GSE33335 and GSE63089 from Gene Expression Omnibus (GEO). Secondly, the DEGs in the two datasets above were identified by using the GEO2R online tool and Venn diagram software. Thirdly, we further analyzed those DEGs containing Gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway by using the Database for Annotation, Visualization and Integrated Discovery (DAVID). Fourthly, a protein-protein interaction (PPI) network and Cytotype Molecular Complex Detection (MCODE) were established for further analysis of the DEGs which enabled us to obtain several core genes. Next, these hub DEGs were imported into the Kaplan-Meier plotter online database to find the correlation between the DEGs and overall survival (OS) in GC patients.. In addition, we also used Gene Expression Profiling Interactive Analysis (GEPIA) to validate the differential expression of DEGs between GC cancer tissues and normal OS tissues. Subsequently, 11 DEGs were found to be eligible, and these were re-analyzed for KEGG pathway enrichment. Finally, three genes [$G_2$/miotic-specific cyclin B1 (CCNB1), polo-like kinases 1 (PLK1), and pituitary tumor-transforming gene-1 (PTTG1)] were identified to be remarkably enriched in the cell cycle pathway, particularly the G1-G1/S phase. Overall, a handful of key genes connected with poor prognosis were discovered via the bioinformatics study, and these may be valid targets for the treatment of GC patients.

We present the following article in accordance with the TRIPOD reporting checklist (available at http://dx.doi.org/10.21037/atm-20-4427).

## Methods

### *DEGs identification*

The GSE33335 and GSE63089 gene expression profiles in GC and normal stomach tissues were acquired from NCBI-GEO (8), which is a free public database of microarray/gene profiles. Microarray data of GSE33335 and GSE63089 separately included 25 GC tissues and 25 matched adjacent noncancerous tissues, along with 45 GC tissues and 45 normal gastric tissues. They were all provided by GPL5175 Platforms. By using GEO2R online tools, the DEGs between GC samples and normal gastric samples were quickly identified. To acquire the co-DEGs among the two datasets, the original data were collated in Venn diagram software. Subsequently, we set $|\log2(\text{fold change})| > 1.2$ and false discovery rate (FDR) <0.01 as the cutoff criteria and define selected DEGs as upregulated genes, while the rest were defined as downregulated genes.

### *GO and pathway enrichment analysis*

Defining genes and their products and identifying genome data or genes' characteristic biological function

can be realized by GO analysis. KEGG, a collection of databases, can manage complex information in genomes, and chemical and biological pathways (9). The biological function of DEGs was identified by DAVID (10), an online bioinformatics tool that can be used to visualize the DEG enrichment of biological process (BP), molecular function (MF), cellular component (CC), and pathways.

### *PPI network and module construction*

Search Tool for the Retrieval of Interacting Genes (STRING) is an online tool that can evaluate complex PPI information (11). The potential correlation between these DEGs were examined by the STRING app in Cytoscape (12). And confidence score ≥0.4 and maximum number of interactors = 0 were set as the cutoff criterion. Furthermore, modules of the PPI network were verified by MCODE in Cytoscape with the following standard: degree cutoff =2, max. depth =100, k-core =2, and node score cutoff =0.2.

### *Survival analysis of hub genes*

For estimating the survival rate, we made the use of the Kaplan-Meier plotter, a website tool based on The Cancer Genome Atlas database, the European Genome-phenome Archive (EGA), and Gene Expression Omnibus (GEO) (affymetrix microarrays only) (13). The log-rank P value and hazard ratio (HR) with 95% confidence intervals (CI) were computed and showed on the plot. To validate these DEGs, GEPIA was applied to map the survival plots based on thousands of samples from TCGA (14).

## Results

### *Identification of DEGs and co-DEGs in the two DEGs in GC*

In the present study, 70 GC tissues and 70 normal gastric tissues in DEGs were extracted from GSE33335 and GSE63089 via GEO2R online tools. Then, we identified the common DEGs in the two datasets by using Venn diagram software. A total of 128 common DEGs were detected, including 85 upregulated genes (logFC >1.2 and FDR <0.01) and 43 downregulated genes (logFC <–1.2 and FDR <0.01) in the GC tissues (*Table 1*, *Figure 1*).

### *GO and KEGG pathway analysis of co-DEGs in GC*

We analyzed all 128 co-DEGs in GSE33335 and GSE63089 and completed the GO analysis via DAVID software. The results demonstrated that co-DEGs were mainly enriched in digestion, collagen fibril organization, extracellular matrix (ECM) organization, wound healing, collagen catabolic process, cell adhesion, positive regulation of cell proliferation, negative regulation of cell proliferation (BP); extracellular exosome, extracellular space, extracellular region, ECM, proteinaceous ECM, apical plasma membrane, collagen trimer (CC); identical protein binding, protein kinase binding, collagen binding, integrin binding, heparin-binding, growth factor activity, ECM binding, platelet-derived growth factor binding (MF) (*Table 2*). For KEGG, the upregulated co-DEGs were mainly involved in the PI3K-Akt signaling pathway, ECM-receptor interaction, protein digestion, absorption, and focal adhesion (*Table 3*).

### *PPI network construction and modular analysis*

The 128 common DEGs and the top 27 core DEGs with the highest node degree were selected by the network analyzer tool. As presented in *Figure 2*, the DEG PPI network was constructed using the 128 common DEGs, while the module of the top 27 core DEGs was established by applying Cytotype MCODE (*Figure 2B,C*).

### *Analysis of hub genes by the Kaplan-Meier plotter and GEPIA*

The survival data of the 27 core genes were calculuated by Kaplan–Meier plotter (http://kmplot.com/analysis) (P<0.05, *Figure 3*), and 25 genes were associated with significantly worse survival in GC patients via GEPIA (*Table 4*). Furthermore, 14 of 25 genes were highly expressed in GC samples compared with normal OS samples (P<0.05; *Table 5* and *Figure 4*).

### *Re-analysis of 14 selected genes via KEGG pathway enrichment*

For a better understanding of the possible pathway of these 14 selected DEGs, we used DAVID to re-analyze KEGG pathway enrichment (P<0.05). Three genes (*CCNB1*, *PLK1*,

**Table 1** All 128 common DEGs were detected from two profile datasets, and 85 upregulated gene, and 43 downregulated genes were included in the GC tissues compared to normal gastric tissue

| Category | Term | Count | % | P value | FDR |
|---|---|---|---|---|---|
| GOTERM_BP_DIRECT | GO:0007586~digestion | 9 | 0.053219 | 1.71E-08 | 2.63E-05 |
| GOTERM_BP_DIRECT | GO:0030199~collagen fibril organization | 7 | 0.041393 | 3.49E-07 | 5.37E-04 |
| GOTERM_BP_DIRECT | GO:0030198~extracellular matrix organization | 11 | 0.065046 | 1.66E-06 | 0.002551 |
| GOTERM_BP_DIRECT | GO:0042060~wound healing | 8 | 0.047306 | 1.84E-06 | 0.002835 |
| GOTERM_BP_DIRECT | GO:0030574~collagen catabolic process | 7 | 0.041393 | 6.91E-06 | 0.010643 |
| GOTERM_BP_DIRECT | GO:0007155~cell adhesion | 14 | 0.082786 | 2.94E-05 | 0.045289 |
| GOTERM_BP_DIRECT | GO:0008284~positive regulation of cell proliferation | 10 | 0.059133 | 0.006950 | 10.190572 |
| GOTERM_BP_DIRECT | GO:0008285~negative regulation of cell proliferation | 9 | 0.053219 | 0.008331 | 12.096443 |
| GOTERM_CC_DIRECT | GO:0005615~extracellular space | 43 | 0.254272 | 2.25E-17 | 2.72E-14 |
| GOTERM_CC_DIRECT | GO:0070062~extracellular exosome | 51 | 0.301578 | 4.97E-11 | 6.02E-08 |
| GOTERM_CC_DIRECT | GO:0005576~extracellular region | 33 | 0.195139 | 3.83E-08 | 4.63E-05 |
| GOTERM_CC_DIRECT | GO:0031012~extracellular matrix | 14 | 0.082786 | 1.53E-07 | 1.85E-04 |
| GOTERM_CC_DIRECT | GO:0005578~proteinaceous extracellular matrix | 12 | 0.070959 | 2.80E-06 | 0.0033832 |
| GOTERM_CC_DIRECT | GO:0016324~apical plasma membrane | 11 | 0.065046 | 3.73E-05 | 0.0451229 |
| GOTERM_CC_DIRECT | GO:0005581~collagen trimer | 7 | 0.041393 | 4.44E-05 | 0.0537706 |
| GOTERM_CC_DIRECT | GO:0016323~basolateral plasma membrane | 8 | 0.047306 | 2.69E-04 | 0.3254291 |
| GOTERM_MF_DIRECT | GO:0005518~collagen binding | 7 | 0.041393 | 3.25E-06 | 0.0041663 |
| GOTERM_MF_DIRECT | GO:0005178~integrin binding | 7 | 0.041393 | 8.15E-05 | 0.1044135 |
| GOTERM_MF_DIRECT | GO:0042802~identical protein binding | 15 | 0.088699 | 5.68E-04 | 0.7252366 |
| GOTERM_MF_DIRECT | GO:0050840~extracellular matrix binding | 4 | 0.023653 | 7.14E-04 | 0.9112294 |
| GOTERM_MF_DIRECT | GO:0048407~platelet-derived growth factor binding | 3 | 0.017739 | 0.002430 | 3.0698870 |
| GOTERM_MF_DIRECT | GO:0008201~heparin binding | 6 | 0.035479 | 0.004777 | 5.9503742 |
| GOTERM_MF_DIRECT | GO:0019901~protein kinase binding | 8 | 0.047306 | 0.014481 | 17.046280 |
| GOTERM_MF_DIRECT | GO:0008083~growth factor activity | 5 | 0.029566 | 0.024856 | 27.564852 |

DEGs, differentially expressed genes; GC, gastric cancer.

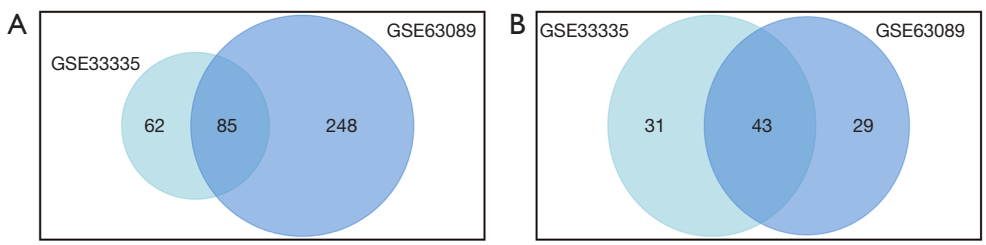



**Figure 1** Authentication of 128 common DEGs in the two datasets (GSE33335 and GSE63089) through Venn diagram software. Different colors represent different datasets. (A) 85 DEGs were upregulated in the two datasets (log2FC >1.2 and FDR <0.01); (B) 43 DEGs were downregulated in the two datasets (log2FC <–1.2 and FDR <0.01). DEGs, differentially expressed genes; FDR, false discovery rate.

**Table 2** Gene ontology analysis of 128 DEGs in gastric cancer

| DEGs | Gene name |
|---|---|
| Up-regulated | *CTSB COL3A1 MUC17 TMPRSS4 EDARADD THY1 RPF2 MYBL2 MACC1 TYMS PLBD1 CTSA TOP2A MMP7 HSP90AB1 CD9 CCNB1 KRT7 ASPN MUC13 CEACAM5 S100A10 PTTG1 PRC1 CXCL1 SPP1 CDC20 CEACAM6 CLDN7 SQLE HIST1H3I COL1A2 PGK1 TFF3 CLDN1 HIST1H4L EPCAM BGN TGFBI UBE2T PI3 PON2 VIL1 KIAA0101 LUM LAPTM4B COL12A1 GPNMB IFITM1 CLRN3 THBS2 HIST1H2BF LAMC2 HIST2H2AB SFRP4 CLDN4 SPARC ARPC1B COL1A1 CDH17 GPRC5A COL6A3 MDK NME1 EIF6 SULF1 CST1 TIMP1 IL32 HIST1H2BM TUBB4B CBX3 CLDN3 CCL20 PLK1 SERPINH1 RRM2 TM4SF1 TMEM176A TPX2 GREM1 CKS2 CDKN3 FN1 HIST1H3B* |
| Down-regulated | *AKR7A3 CHIA KCNE2 ANXA10 GKN2 MT1G ESRRG AQP4 SLC5A5 MFSD4A GIF CKB CXCL17 CA9 CA2 LIPF CPA2 PDILT MT1M PSCA AKR1B15 TFF1 PGC DPCR1 GHRL PDIA2 VSIG1 ATP4A CCKAR CLIC6 GKN1 MT1F FBP2 CHGA TMED6 LIFR TFF2 SST GSTA5 ATP4B VSIG2 PGA5 SLC26A9* |

DEGs, differentially expressed genes; FDR, false discovery rate.

**Table 3** KEGG pathway analysis of diferentially expressed genes in gastric cancer

| Pathway ID | Name | Count | % | P value | Genes |
|---|---|---|---|---|---|
| hsa04512 | ECM-receptor interaction | 8 | 0.047 | 2.55E-05 | *COL3A1, COL6A3, COL1A2, LAMC2, COL1A1, THBS2, SPP1, FN1* |
| hsa04974 | Protein digestion and absorption | 7 | 0.041 | 2.51E-04 | *COL3A1, COL6A3, COL1A2, CPA2, COL12A1, COL1A1, PGA5* |
| hsa04510 | Focal adhesion | 8 | 0.047 | 0.004729 | *COL3A1, COL6A3, COL1A2, LAMC2, COL1A1, THBS2, SPP1, FN1* |
| hsa04971 | Gastric acid secretion | 5 | 0.029 | 0.006313 | *ATP4A, ATP4B, KCNE2, CA2, SST* |
| hsa04978 | Mineral absorption | 4 | 0.023 | 0.009919 | *MT1M, SLC26A9, MT1G, MT1F* |
| hsa05322 | Systemic lupus erythematosus | 6 | 0.035 | 0.011454 | *HIST2H2AB, HIST1H2BM, HIST1H4L, HIST1H2BF, HIST1H3B, HIST1H3I* |
| hsa05146 | Amoebiasis | 5 | 0.029 | 0.022457 | *COL3A1, COL1A2, LAMC2, COL1A1, FN1* |
| hsa04151 | PI3K-Akt signaling pathway | 9 | 0.053 | 0.023099 | *HSP90AB1, COL3A1, COL6A3, COL1A2, LAMC2, COL1A1, THBS2, SPP1, FN1* |

KEGG, Kyoto Encyclopedia of Genes and Genomes; ECM, extracellular matrix.

and *PTTG1*) were found to be significantly enriched in the cell cycle pathway, especially in the G1-G1/S phase (*Table 6* and *Figure 5*).

## Discussion

For identifying more useful prognostic biomarkers in GC cancer, bioinformatical methods based on two profile datasets (GSE33335 and GSE63089) were applied in this study, which included 70 GC specimens and 70 normal specimens. A total of 128 commonly changed DEGs (|logFC| >2; adjusted P value <0.05) including 85 upregulated (Log FC >0) and 43 downregulated DEGs were identified (Log FC <0) via GEO2R and Venn diagram software. Next, we used the DAVID online tool to analyse the GO and KEGG pathways of the co-DEGs. The analysis results demonstrated that for KEGG, co-DEGs were particularly enriched in digestion, collagen fibril organization, ECM organization, wound healing, collagen catabolic process, cell adhesion, positive regulation of cell proliferation, negative regulation of cell proliferation (BP); extracellular exosome, extracellular space, extracellular region, ECM, proteinaceous ECM, apical plasma membrane, collagen trimer (CC); in identical protein binding, protein kinase binding, collagen binding, integrin binding, heparin-binding, growth factor activity, ECM binding, platelet-derived growth factor binding (MF). For KEGG, the upregulated co-DEGs were particularly involved in the PI3K-Akt signaling pathway, ECM-receptor interaction, protein digestion, absorption, and focal

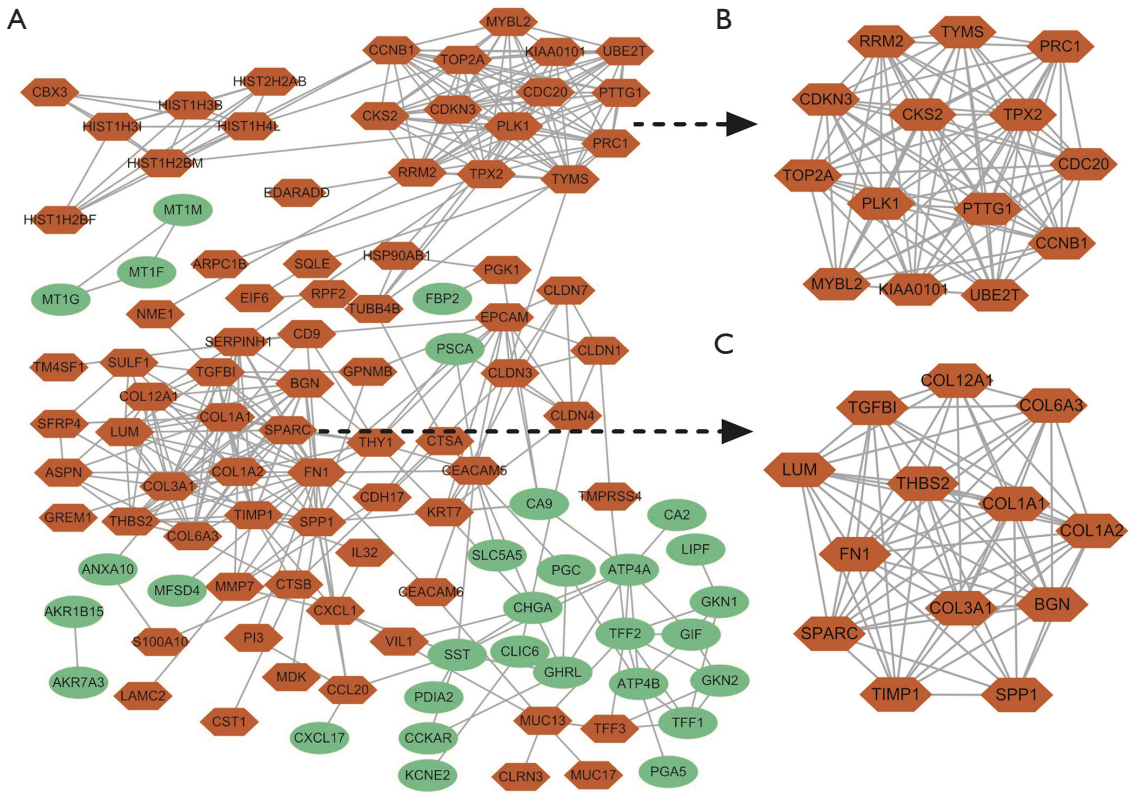


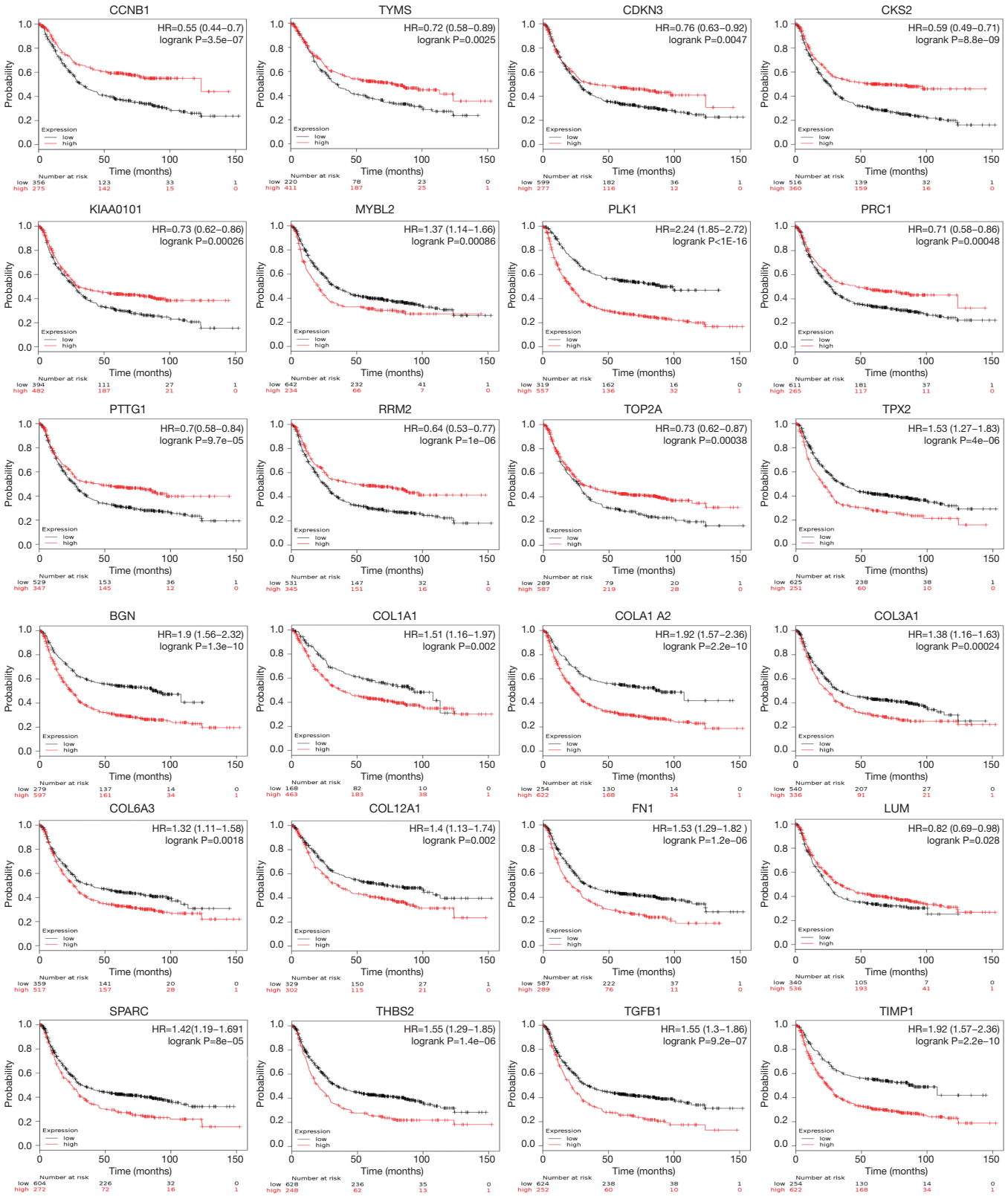

**Figure 2** Common DEG PPI network constructed by STRING online database and module analysis. (A) There were a total of 128 DEGs in the DEG PPI network complex. The nodes represent proteins, and the edges represent the interaction of proteins. The red boxes indicated upregulated genes, while the green boxes indicate downregulated genes. (B,C) Module analysis via Cytoscape software (degree cut-off =2, node score cut-off =0.2, k-core =2, and max. depth =100) and two important modules were built based on the network. DEGs, differentially expressed genes; PPI, protein-protein interaction.

adhesion.

A DEG PPI network was also constructed, and 27 core genes were screened by Cytotype MCODE analysis. Furthermore, through Kaplan-Meier plotter analysis, 25 of 27 genes were found to be associated with significantly worse survival. In validating these 25 genes with GEPIA analysis, 14 genes showed high expression in GC samples compared with normal samples (P<0.05). Finally, the 14 genes were re-analyzed via DAVID for KEGG pathway enrichment, and then 3 genes (CCNB1, PLK1, and PTTG1) were found to be significantly enriched in the cell cycle pathway, particularly the G1-G1/S phase (P<0.05). These may be regarded as new effective targets for improving the prognosis of GC patients.

CCNB1 is a member of the cyclin B family, and plays a critical role in cells inspecting into or out of M phase in the cell cycle. It is a monitoring protein involved in mitosis and is primarily expressed during the G2/M phase (15). Over the past decades, a large amount of research has demonstrated that CCNB1 is overexpressed in various cancers with poor prognosis, including breast cancer (16), colorectal cancer (17), oral cancer (18), and GC. It was reported that the suppression of CCNB1 by Huang Lian treatment could suppress tumor cell growth in GC by preventing cells from going into the M phase. Moreover, the research by Yasuda *et al.* shows that the overexpression of CCNB1 occurs in GC and primarily in the early stage. They then further confirmed that high CCNB1 overexpression usually occurs before tumor cells acquire immortalization ability (19). As can be seen, CCNB1 is a well-studied biomarker of GC and is valuable for the prevention and evaluation of therapeutic effects.

PLK1, which belongs to the family of serine/threonine protein kinases, is widespread in eukaryotic cells and has



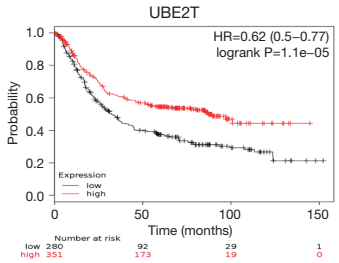



**Figure 3** The prognostic information of the 27 core genes. The online Kaplan-Meier plotter tool was used to identify the prognostic information of the 27 core genes, with 25 of 27 genes being associated with a significantly worse survival rate (P<0.05).

**Table 4** The prognostic information of the 27 key candidate genes

| Category | Genes |
|---|---|
| Genes with significantly worse survival (P<0.05) | *CDKN3, UBE2T, MYBL2, PLK1, CKS2, TPX2, TYMS, CCNB1, PTTG1, RRM2, KIAA0101, TOP2A, PRC1, FN1, COL12A1, COL1A1, LUM, COL3A1, TIMP1, COL1A2, TGFBI, SPARC, THBS2, BGN, COL6A3* |
| Genes without significantly worse survival (P>0.05) | *CDC20, SPP1* |

**Table 5** Validation of 25 genes via GEPIA

| Category | Genes |
|---|---|
| Genes with high expressed in GC (P<0.05) | *BGN, CCNB1, CDKN3, CKS2, COL1A1, KIAA0101, MYBL2, PLK1, PTTG1, RRM2, THBS2, TOP2A, TPX2, UBE2T* |
| Genes without high expressed in GC (P>0.05) | *TYMS, PRC1, FN1, COL12A1, LUM, COL3A1, TIMP1, COL1A2, TGFBI, SPARC, COL6A3* |

GEPIA, Gene Expression Profiling Interactive Analysis; GC, gastric cancer.

been investigated more intensively than the other four PLKs (20,21). PLK1 takes part in cell mitosis and plays a pivotal role in multiple steps, including $G_2$-M transition, centrosome maturation, bipolar spindle formation, chromosome segregation, DNA replication, and spindle formation (22,23). Decades ago, several studies revealed that the overexpression of PLK1 was closely related to occurrence and the development of malignant tumors (24), including those of GC (25), breast cancer (26), ovarian carcinoma (27), melanoma (28), glioma (29), and renal cancer (30). Furthermore, Wilko *et al.* demonstrated that (31) PLK1 was overexpressed in roughly half of all gastric carcinomas, and was associated with worse prognosis. Interestingly, in the intestinal metaplasia of normal gastric mucosa, PLK1 has also been found to be expressed or overexpressed. In a relatively new study, we showed that CIP2A, an inhibitor of protein phosphatase 2A, plays a crucial role in facilitating the stability and activity of PLK1 during mitosis by interacting directly with the polo-box domain of PLK1. Thus, the findings above suggest that the CIP2A-Plk1 complex may serve as a potential prognostic marker for poor survival cancer patients. Moreover, small molecules interfering with CIP2A-Plk1 binding could be effective as antimitotic drugs for cancer therapy (32).

PTTG1 is a transcription factor which functions in various physiological events, including transcriptional activity, neovascularization, and cell senescence, and can also participate in cell division, chromosome stability, and DNA repair by encoding regulatory proteins (33,34). After being discovered first in rat pituitary tumor (35), PTTG1 was subsequently reported to be overexpressed in GC (36,37), pituitary adenomas (38), ovarian carcinoma (38), colon carcinoma (39), lung cancer (40), and breast cancer (41). Xu *et al.* reported that GC tissues expressed a higher level of PTTG1 than adjacent normal tissues. Interestingly, they also found that in GIN, a precancerous lesion, PTTG1

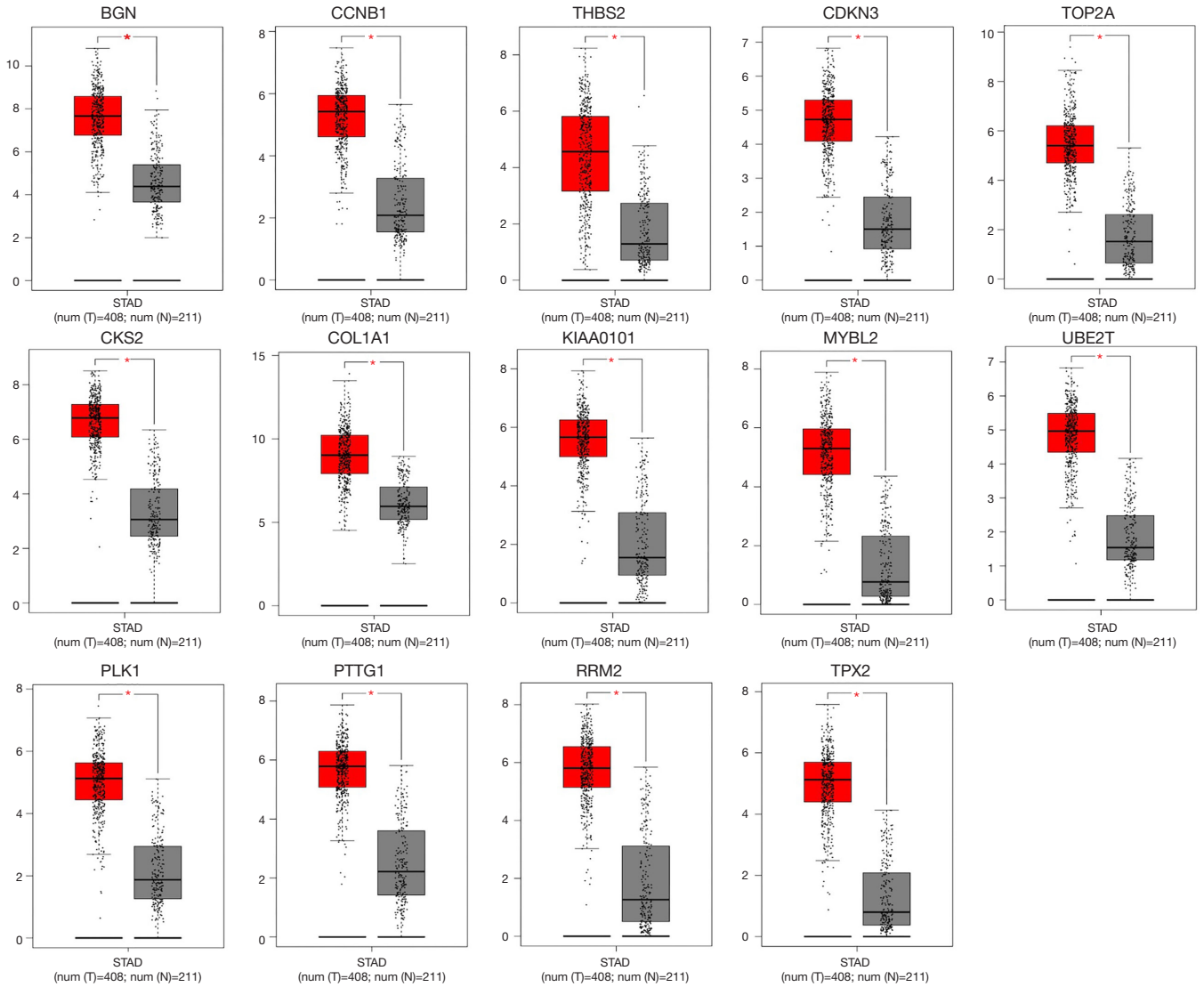



**Figure 4** Fourteen genes in gastric cancer patients were significantly expressed as compared to healthy controls. To further identify the difference of gene expression level between gastric cancer patients and healthy controls, 25 genes that were related to poor prognosis were analyzed by the GEPIA website: 14 of 25 genes had significant expression levels in gastric cancer specimens compared to the normal specimens ($Log_2FC \geq 3$, *P<0.001). Red color represents tumor tissues, and grey color represents normal tissues. GEPIA, Gene Expression Profiling Interactive Analysis.

**Table 6** Re-analysis of 14 selected genes via biological pathway enrichment

| Term | Name | Count | % | P value | Genes |
|---|---|---|---|---|---|
| cfa04110 | Cell cycle | 3 | 21.4 | 0.007 | *CCNB1, PLK1, PTTG1* |

protein expression was significantly higher than para-carcinoma tissues (42,43). Although the specific regulatory mechanisms of PTTG1 in GIN or GC are relatively poorly understood, we can conclude that significant PTTG1 overexpression, both at the mRNA and protein levels, occurring in GC cells *in vitro* and *in vivo*, might hold value

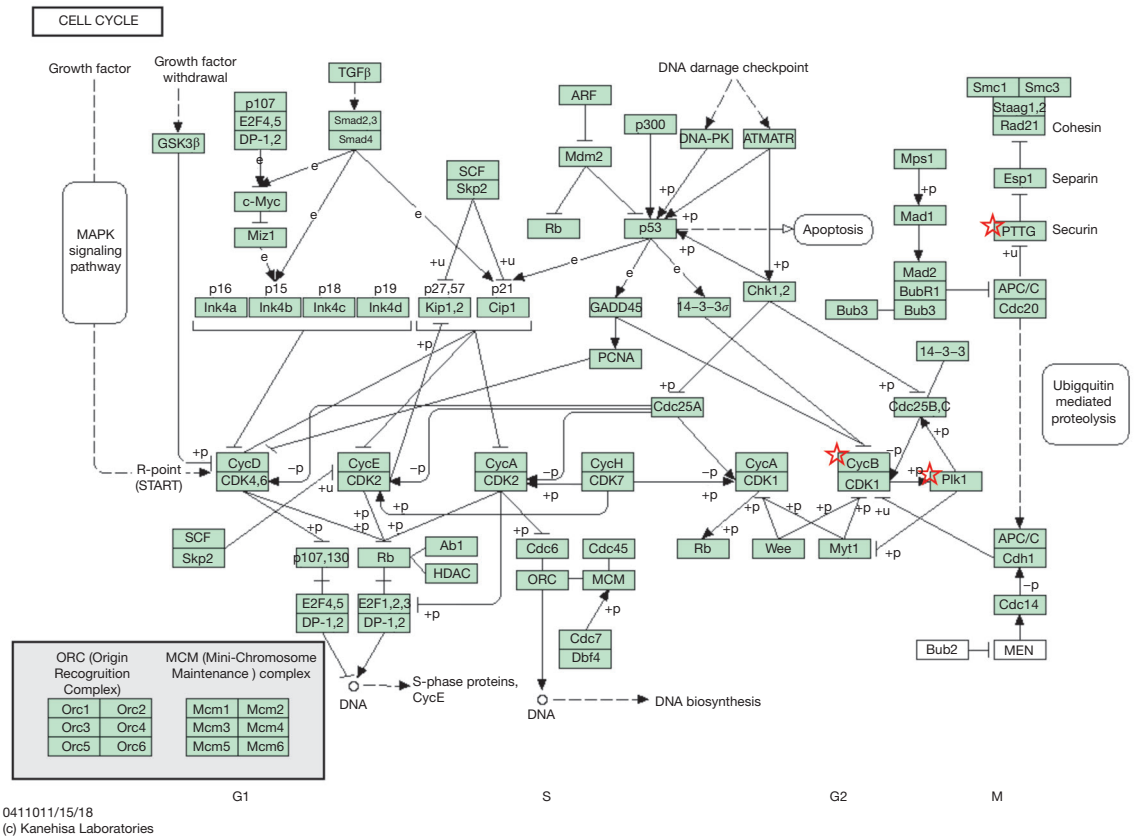



**Figure 5** Re-analysis of 14 selected genes using KEGG pathway enrichment. The 114 highly expressed genes in gastric cancer tissues with poor prognosis were re-analyzed by KEGG pathway enrichment. Three genes (CCNB1, PLK1, and PTTG1) were significantly enriched in the cell cycle pathway. Plk1 means PLK1, PTTG means PTTG1, CycB means CCNB1. KEGG, Kyoto Encyclopedia of Genes and Genomes; CCNB1, G2/miotic-specific cyclin B1; PLK1, polo-like kinases 1; PTTG1, pituitary tumor-transforming gene-1.

in GC diagnosis and therapy.

A growing number of studies have implicated these three genes in the emergence and progression of various types of cancers. Unfortunately, few studies have attempted to elaborate the mechanism of action and the precise role of the three genes in GC cancer. We thus hope that the data acquired from our research of GC cancer may offer greater focus to the direction of future studies.

## Conclusions

We identified three DEGs (CCNB1, PLK1, and PTTG1) between GC tissues and normal tissues in our bioinformatics analysis study on the base of datasets GSE33335 and GSE63089. Results showed that these three genes could play critical roles in the progression of GC. However, these predictions should be verified by a series of experiments in the future. Overall, this data may provide valuable information and direction for future investigation into the potential biomarkers and biological mechanisms of GC.

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## Footnote

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