Identification of Function Genes regulating Gastric Cancer progression using Integrated Bioinformatics Analysis

Kun Yu
Shanghai Dahua Hospital
Yao Xiao
Shanghai Institute of Measurement and Testing Technology
Qiang Yao
Shanghai Dahua Hospital
Haiyang Qian
Shanghai Dahua Hospital
Xin Pan
Shanghai Dahua Hospital
Gang Wang
Shanghai Dahua Hospital
Qiong Chen
Shanghai Dahua hospital
Dong Zhang
Shanghai University of Traditional Chinese Medicine Yueyang Hospital of Integrated Traditional Chinese Medicine and Western Medicine
Ke Mei (lunasanger@126.com)
Shanghai Dahua Hospital https://orcid.org/0000-0002-6958-779X

Research Article

Keywords: Gastric cancer, Bioinformatics analysis, Differentially expressed gene, Protein–protein interaction network, cisplatin resistance

Posted Date: April 1st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1343178/v1

Abstract

Gastric cancer(GC)is the most frequent cause of cancer with poor prognosis. Treatment for GC remains unchanged in the past few years. This study was done to investigate the potential therapeutic targets and related regulatory biomarkers of Gastric cancer. We obtained the public GC transcriptome sequencing dataset from the GEO database. The datasets contains 348 GC tissues and 141 healthy tissues. In total, 251 DEGs were identified, containing 187 down-regulated genes and 64 up-regulated genes. The DEGs' enriched functions and pathways include Progesterone-mediated oocyte maturation, cell cycle, and oocyte meiosis, Hepatitis B, and Hippo signaling pathway. Survival analysis showed that BUB1, MAD2L1, CCNA2, CCNB1, and BIRC5 might be associated with the regulation the cell cycle phase mitotic spindle checkpoint pathway. We selected 26 regulated genes with the PPI networks' aid analyzed by the Molecular Complex Detection. Then we focused on three critical gene, which is high expressed in gastric cancer, while negatively related to patients survival. Furthermore, we found that knockdown either BIRC5, TRIP13 or UBE2C significantly inhibited cell proliferation, induced cell apoptosis. Knockdown of BIRC5, TRIP13 or UBE2C increased cellular sensitivity to cisplatin. Together, our present study identified significantly upregulated genes with worse prognosis in GC using integrated bioinformatics methods.

Introduction

Gastric cancer remains the common cause of cancer death globally, although the mortality of gastric cancer has declined obviously in the past year[1]. Even though tremendous efforts have been made to analyze the pathogenesis, the limited knowledge of GC's exact molecular mechanisms is still unknown. It was reported that several prognostic potential biomarkers, although have been exploited, the precise pathogenic mechanism of GC remains to be illustrated[2, 3]. It is essential to investigate the variations of global gene expression to enhance the efficacy of the treatment and better assist us in understanding the gastric cancer's pathogenesis. As a mature technology, gene chip is widely used in modern medicine[4]. It can detect differentially expressed genes quickly, which are stored in public databases conveniently. Therefore, many related work on GC have been published in recent years[5]. Genetic factors were revealed to play an important role in the susceptibility to GC.

This paper chose the GSE13911, GSE66229, and GSE79973 datasets from the Gene Expression Omnibus (GEO). The expressed genes (DEGs) in the three datasets above were available from the GEO2R online tool and Venn diagram software. We used the Database for Annotation, Visualization, and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov) to analyze the screened DEGs containing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyze. Then, we used the protein-protein interaction (PPI) network DEGs to analyze the DEGs and screen the PPI network modules in GC as well.

We applied Cytotype Molecular Complex Detection (MCODE) to screen the key gene modules and co-expression networks to seek the relative significance in the three datasets above. Among these DEGs, 26 genes had significant difference in the PPI network. Subsequently, the 26 core DEGs were generated by the Kaplan Meier plotter online database (http://www.kmplot.com) for the crucial prognostic information (P <

0.05). Also, we determined the DEGs expression in Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn) ($P < 0.05$) between Gastric cancer tissues and normal gastric tissues.

We qualified 22 DEGs based on these data. After that, the 22 DEGs were re-analyzed for KEGG pathway enrichment. Subsequently, based on survival analysis, three DEGs (BIRC5, TRIP13 and UBE2C) were important related genes and played key part in cellular pathways. Thus, we found the underlying gene pathways and potential molecular mechanisms. And the results may contribute to the treatment, diagnosis, and prevention of GC. Cellular experiments also revealed that targeting these three genes inhibited proliferation of gastric cancer cell lines, overcome the resistance to cisplatin, which provide novel therapeutic targets for gastric cancer.

Materials And Methods

Microarray data information

The date expression profiles of Gastric cancer and healthy gastric tissues in the GSE13911, GSE66229, and GSE79973 datasets were available from the NCBI-GEO website which were all done on the GPL570 platform, an open and known database of microarray/gene profiles. Microarray data of GSE13911, GSE66229, and GSE79973 were all based on Affymetrix HGU 133 Plus 2 microarray (Affymetrix, Inc., Santa Clara, CA, USA).

In GSE13911, there were 69 samples from 38 GC patients and 31 normal people; GSE66229 contains microarray data from healthy controls and Gastric cancer disease. There were 400 samples in GSE66229, including 300 GC samples and 100 control samples. GSE79973 contains data from 10 cognitively healthy controls and 10 Gastric cancer disease patients.

Data processing of DEGs

DEGs between Gastric cancer tissues and healthy tissues were identified via GEO2R online tools[6] with adjusted P-value < 0.05 and |log FC| > 0.5. In the three datasets above, the original results were selected by Venn diagram software online to explore the common DEGs. The DEGs were distinguished by log FC as follows: the genes with log FC fold change > 0 were up-regulated, while the genes with log FC fold change < 0 were down-regulated.

Gene ontology and pathway enrichment analysis

Gene ontology (GO; http://www.geneontology.org) analysis is a commonly used approach for the unification of biology that offers information about gene product function using ontologies to represent biological knowledge[7]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a large-scale database[8] including drugs, genomes, diseases, chemical materials, and biological pathways, and so on. The Database for Annotation, Visualization and Integrated Discovery (DAVID) is a useful bioinformatics tool designed to check a large number of gene or protein functions online[9]. We used DAVID to identify GO categories and pathways ($P < 0.05$).

PPI network and module analysis

Based on the STRING information (Search Tool for the Retrieval of Interacting Genes) database[10], the PPI network was constructed. Then, the STRING database was visualized using Cytoscape software version, a popular open-source software tool from the network: www.Cytoscape.org[11], to identify the prognostic correlation among the DEGs above, we set the confidence score ≥ 0.4(modest confidence). The MCODE program in Cytoscape was also used to explore the PPI network modules (we set: Node score cut off = 0.2, degree cut off = 2, k-core = 2, and max. Depth from seed = 100).

Survival analysis and RNA sequencing expression of core genes

Kaplan Meier-plotter (KM plotter, http://kmplot.com/analysis/) was widely used to assess the effect of plenty of genes on survival based on EGA, TCGA database, and GEO. The log-rank P-value and hazard ratio (HR) with 95% confidence intervals(CI) were computed and showed on the plot[12]. To validate these DEGs, we applied the GEPIA website to analyze RNA sequencing expression data based on thousands of samples from the GTEx projects and TCGA[13].

Patient sample and RT-PCR detection

We performed RT-PCR analysis from 15 pairs of patient samples including tumor and the adjacent normal tissues in accordance with the ethic permission approved in Dahua hospital. RNA was extract in Trizol methods as previously described. After then, cDNA was synthesized with a reverse transcription kit (Takara, China). Expression level of BIRC5, TRIP13 and UBE2C genes were detected with a SYBR real-time PCR kit from Takara. The primers used for detecting these three genes were purchased from Thermal Fisher Co.; GAPDH was used as a control.

Cells and transfection

Human gastric cancer cell lines MKN-28 were purchased from the American Type Culture Collection (ATCC). All cells were maintained under 37 centigrade in 5% CO_2 humid chamber, in RPMI1640 medium (PAA) supplemented with 10% fetal bovine serum (FBS, Gibico) and streptomycin and penicillin (10mg/mL). For knockdown of BIRC5, TRIP13 and UBE2C genes, specific shRNA was transfected into MKN-28 cells with a lipo3000 transfection reagent. Transfected cells were used for next experiments.

CCK-8 assay and Apoptosis assay

For cell proliferation, we used a CCK-8 assay in cells with specific gene knockdown. Briefly, 5000 cells were seeded in each well of 96 well plates. At 24, 48 and 72h after plating, cells were stained with CCK8 solution from CCK-8 kit (Beyotime, China) and detected under OD570 wavelength. Cell apoptosis assay was performed by using a Annexin V/PI double staining kit according to manufacture's instructions.

Colony formation assay

For colony formation assay, cells were seeded in 60mm dishes in indicated numbers of 400, 800, 1600, 3200 per dishes. 24h later, cells were treated with cisplatin at the concentration of 0, 2, 4, 8μM. After treatment, cells were returned to incubator for additional 10 days. Colonies were counted after stained with ultraviolet staining solution.

Statistics analysis

As shown in Figure 1: The flow diagram of this study demonstrate the raw exprimental data were processed via several software. The moderate t-test was applied to identify DEGs, And Fisher's Exact test was used to analyze GO and KEGG annotation enrichments[14].

Results

Identification of DEGs in Gastric cancer disease

In our current study, there were 348 GC tissues and 141 healthy tissues. With the help of GEO2R online tools, we obtained 5599, 2755, and 2473 DEGs from the three datebases: GSE13911, GSE66229, and GSE79973, respectively. Among the three datasets, the common DEGs were identified via Venn diagram software. The findings revealed that althogether 251 common DEGs were explored, containing 187 and 64 genes which controlling down and up related genes in the Gastric cancer tissues (Table 1 & Fig. 2).

Table 1
All 251 commonly differentially expressed genes (DEGs) were detected from three profile datasets, including 187 down-regulated genes and 64 up-regulated genes in the Gastric cancer tissues and normal gastric tissues.

DEGs	Genes Name
up-regulated	*IGF2BP3 CCNB1 LOC100129518///SOD2 COL1A1 BIRC5 ADAMTS2 KLHL7 KIF14 MAD2L1 BICD1 SPP1 FAM72A///FAM72D///FAM72B///FAM72C NDC80 CCNA2 NUF2 UBE2T TEAD4 ECT2 CDH3 MTFR2 PRC1 LRP8 CEP55 CEMIP DDIAS S100A2 WISP1 AJUBA CLDN1 APOC1 NEK2 ESM1 PLAU DEPDC1B HMGB3 CXCL8 IL13RA2 HOXA10 KIF2C CDCA7 BUB1 TRIP13 DUXAP10 LOC101928195///LOC100996643///MTHFD1L TMEM158 ASPM INHBA ATAD2 KNL1 STIL UBE2C ULBP2 TOP2A WDR72 DTL MEST KIF20A COL10A1* *PMEPA1 HOXC6 CTHRC1 CDKN3 LINC01296///DUXAP10 CENPF*
down-regulated	*BTG2 ATP5F1 PDE1C MAOA ZNF385B PWP2 ALAD ACADL PIR-FIGF///FIGF ZBTB20 HDHD2 NTN4 SERP1* *SYNJ2BP-COX16///SYNJ2BP PPID SCNN1B KLHDC2 ADCYAP1R1 UBE2QL1 FAM13A ADHFE1 SLC25A4 LOC100506870///LOC283140 MYOC GIF SYNE1 GNG7 VSTM2A ZSCAN31 PRDM5 EFCC1 STAM2 TLE4 SH3GL2* *CKMT2 BBIP1 KCNJ16 SLC26A7 ADAMTSL1 KIAA2022 PCBP1-AS1 FAHD2CP SLC16A7 FMO4 PGRMC2 ATP4 ACD36 FGD4 MAL AQP4 ESRRG LYRM5 CPA2 ABCA8 EFCAB14 DBT SLC1A2 TPCN2 TCEB3 USP53 ARRDC4 TMEM100 CREBL2 SPINK2 CNTN3 PRDM11 ETNPPL RGS5 LOC101927263 PACRG RCCD1 UBL3 CYB5R1 LINC01105 ADGRL3 ATP4B TMEM116 SMIM14 6-Mar ZNF626 LOC728730 ENPP6 CIRBP ECHDC2 GPX3 KCNMB2 THSD4 HDC MTERF2 SAR1B TRIM50 FAM214A MITF TRIM74///TRIM73 RNF14 CLTA IGH KL BDH2 APOBEC2 DGKD RPS6KA6 MFSD4A ALDH6A1 PHYKPL NEDD4L PDGFD KIT GCNT2 MIR29C///MIR29B2 SIK2 DMXL1 CWH43 PGA4///PGA3///PGA5 PDK4 GFRA2 TRIP11 HACD1 GPR155 NR3C2 MUT UMAD1 OPCML LOC100505501 PLIN5 RNASE1 MYZAP SMIM5 PLPP3 ACACB GRIA4 BAALC RPRM CYFIP2 FBXL13 SPC24 YIF1B RGMB SMDT1 FBP2 FAM150B CADM2 LINC00849///SLC25A16 MAGI3 CHGA WIPF3 TOM1L2 VAPA RAB11B-AS1 ADH7 ASPA PDILT C14orf159 TXNL1 LINC00982 CCKBR ADRB2 GRIA3 NTRK3 SLC7A8 SCARA5 ACADSB CPEB2 SLC2A12 LIFR STX12 FNDC5 PCAT18 HADH 2-Mar GSTA3 SIDT2 CHIA ZEB2 METTL7A KCNE2 RAB27A TAPT1 LOC101926959 SIGLEC11 LOC101929219///C1orf186 DNER GAB1 C21orf58 KAT6B DYX1C1-CCPG1///CCPG1*

Identification and function of DEGs in GC

Associated with GO terms, the genes of up-regulated and down-regulated DEGs were enriched in the cell division, mitotic nuclear division, and mast cell cytokine production. The markly up-regulated and down-regulated DEGs in biological process (BP) was cell division, mitotic nuclear division, and mast cell cytokine production. In addition to the enrichment in mitotic metaphase plate congression, the up-regulated DEGs were significantly enriched in the BPs of cell division. In contrast, the down-regulated DEGs are significantly enriched in the metabolic process.

Gene ontology and pathway enrichment analysis

We performed functional and pathway enrichment analyses to investigate the biological classification of DEGs via DAVID. There were three categories in GO analysis: Biological process (BP), cellular component (CC), and molecular function (MF) GO. In biological processes-associated category showed by GO pathway enrichment analysis, the genes were significantly involved in nuclear chromosome segregation, sister chromatid segregation, cell division, cell cycle process, mitotic cell cycle process and down-regulated DEGs in organic acid catabolic process, carboxylic acid catabolic process, small molecule catabolic process, lipid modification, fatty acid catabolic process, fatty acid oxidation (Table 2). Molecular function (MF) of DEGs were significantly enriched in identical protein binding, microtubule binding, enzyme binding, tubulin binding, cytoskeletal protein binding protein kinase binding, cofactor binding, coenzyme binding, potassium channel regulator activity, ligand-gated ion channel activity, ligand-gated channel activity (Table 2).Changes in the cell components (CCs) of DEGs were mainly enriched in the condensed chromosome kinetochore, condensed chromosome, centromeric region kinetochore, condensed chromosome, chromosome, centromeric region, midbody (Table 2& Fig. 3).

Table 2
Gene ontology analysis of differentially expressed genes in GC

Expression	Category	Term	Count	%	p-Value	FDR
Up-regulated	GOTERM_BP_DIRECT	GO:0051301 ~ cell division	14	14.2	1.2E-10	1.74 E-07
	GOTERM_BP_DIRECT		11	11.16	9.7E-09	1.4 E-05
Down-regulated	GOTERM_BP_DIRECT	GO:0007067 ~ mitotic nuclear division	8	8.1	5.5E-08	7.93 E-05
	GOTERM_BP_DIRECT		5	5	8.3E-05	0.11983
	GOTERM_BP_DIRECT	GO:0007062 ~ sister chromatid cohesion	4	4	2.7E-04	0.38443
	GOTERM_BP_DIRECT	GO:0007059 ~ chromosome segregation	4	4	5.8 E-04	0.831662
	GOTERM_CC_DIRECT		9	9.13	6.26E-09	7.16 E-06
	GOTERM_CC_DIRECT	GO:0007080 ~ mitotic metaphase plate congression	8	8.12	9.6 E-09	1.10E-05
	GOTERM_CC_DIRECT		6	6.09	5.35E-06	0.00612
	GOTERM_CC_DIRECT	GO:0000910 ~ cytokinesis	5	5.07	3.00E-05	0.034353
	GOTERM_CC_DIRECT		3	3.04	5.74E-05	0.065715
	GOTERM_CC_DIRECT	GO:0030496 ~ midbody	5	5.07	3.75E-05	0.428069
	GOTERM_MF_DIRECT	GO:0000777 ~ condensed chromosome kinetochore	10	10.15	6.46E-04	0.747165
	GOTERM_MF_DIRECT		41	41.60	0.00139	1.596840
	GOTERM_MF_DIRECT	GO:0000776 ~ kinetochore	4	4.06	0.02218	22.56086
	GOTERM_MF_DIRECT		5	5.07	0,02801	22.92955
	GOTERM_MF_DIRECT	GO:0000775 ~ chromosome, centromeric region	3	3.04	0.03028	28.10169
	GOTERM_BP_DIRECT		8	3.04	0.00243	3.70122
	GOTERM_BP_DIRECT	GO:0000942 ~ condensed nuclear chromosome outer kinetochore	4	1.52	0.00657	9.731282
	GOTERM_BP_DIRECT		3	1.14	0.01165	16.64097
	GOTERM_BP_DIRECT	GO:0000922 ~ spindle pole	3	1.14	0.01287	18.2266
	GOTERM_BP_DIRECT		6	2.28	0.01574	21.83896
	GOTERM_BP_DIRECT	GO:0042802 ~ identical protein binding	2	0.76	0.01731	23.7548
	GOTERM_CC_DIRECT		12	4.56	1.14E-04	0.139087
	GOTERM_CC_DIRECT	GO:0005515 ~ protein binding	22	8.36	0.00442	5.251997
	GOTERM_CC_DIRECT		22	8.36	0.00873	10.12515
	GOTERM_CC_DIRECT	GO:0016887 ~ ATPase activity	5	1.9	0.02325	24.90296
	GOTERM_CC_DIRECT	GO:0004842 ~ ubiquitin-protein transferase activity	47	17.9	0.02444	26.00801
	GOTERM_CC_DIRECT		34	12.9	0.03132	32.1178

GOTERM_MF_DIRECT	GO:0003777 ~ microtubule motor activity	4	0.47	0.004676	6.175478
GOTERM_MF_DIRECT	GO:0034220 ~ ion transmembrane transport	3	2.17	0.021778	25.87697
GOTERM_MF_DIRECT	GO:0006635 ~ fatty acid beta-oxidation	2	2.52	0.0252	29.32705
GOTERM_MF_DIRECT	GO:0009083 ~ branched-chain amino acid catabolic process	3	2.65	0.026548	30.6453
GOTERM_MF_DIRECT	GO:0030318 ~ melanocyte differentiation	4	3	0.033459	37.04963
	GO:0008152 ~ metabolic process				
	GO:0032762 ~ mast cell cytokine production				
	GO:0005759 ~ mitochondrial matrix				
	GO:0005739 ~ mitochondrion				
	GO:0005887 ~ integral component of plasma membrane				
	GO:0043235 ~ receptor complex				
	GO:0005886 ~ plasma membrane				
	GO:0070062 ~ extracellular exosome				
	GO:0015459 ~ potassium channel regulator activity				
	GO:0005496 ~ steroid binding				
	GO:0008900 ~ hydrogen:potassium-exchanging ATPase activity				
	GO:0000062 ~ fatty-acyl-CoA binding				

	GO:0004971 ~ AMPA glutamate receptor activity

Table 3
Pathway Enrichment Analysis of Common Genes Function in Gastric Cancer

Pathway ID	Name	Count	%	p-valve	Genes
hsa04914	Progesterone-mediated oocyte maturation	4	6.67	0.001636	CCNB1, MAD2L1, BUB1, CCNA2
hsa04110	Cell cycle	4	6.67	0.0044859	CCNB1, MAD2L1, BUB1, CCNA2
hsa04114	Oocyte meiosis	3	5	0.0369046	CCNB1, MAD2L1, BUB1
hsa05161	Hepatitis B	3	5	0.0597128	CCNA2, CXCL8, BIRC5
hsa04390	Hippo signaling pathway	3	5	0.0641468	TEAD4, BIRC5, AJUBA
hsa05202	Transcriptional misregulation in cancer	3	5	0.0765009	HOXA10, CXCL8, PLAU

PPI network and modular analysis

Totally, containing 193 nodes and 633 edges, 251 DEGs were imported into the DEGs PPI network complex, and 187 down-regulated and 64 up-regulated genes were included (Fig. 4a). The 187 down-regulated DEGs were not contained in the DEGs PPI network (Fig. 4a). Then we made use of Cytotype MCODE to gain further results: The outcomes showed that among the 64 nodes there were 26 central nodes of up-regulated genes (Fig. 4b).

KM plotter and GEPIA datebase analysis

We identified the survival data of 26 core genes via Kaplan Meier plotter(http://kmplot.com/analysis/). Among these genes, 24 genes showed a significant worse survival, while 2 had no significant ($P < 0.05$, Table 4 & Fig. 5, Fig. S1). Then, between cancerous and normal genes, GEPIA was used to dig up the 24 gene expression level. Contrasted to normal stomach samples, results revealed that 22 of 24 genes reflected highly enriched in GC samples. ($P < 0.05$, Table 5 & Fig. 5, Fig. S2)

Table 4
The prognostic information of the 26 key candidate genes

Category	Genes
Genes with significantly worse survival (P < 0.05)	*ASPM ATAD2 BIRC5 BUB1 CASC5 CCNA2 CCNB1 CDKN3 CENPF CEP55DEPDC1B DTL ECT2KIF14 MAD2L1 NDC80 NEK2 NUF2 PRC1 STILTOP2A TRIP13 UBE2C UBE2T*
Genes with significantly worse survival (P > 0.05)	*RAB6KIFL KIF2C*

Table 5
Vadidation of 22 genes via GEPIA

Category	Genes
Genes with high expressed in GC (P < 0.05)	*ASPM ATAD2 BIRC5 BUB1 CASC5 CCNA2 CCNB1 CDKN3 CENPF CEP55DEPDC1B DTL ECT2KIF14 MAD2L1 NDC80 NEK2 NUF2 PRC1 STILTOP2A TRIP13 UBE2C UBE2T*
Genes without high expressed in OC (P > 0.05)	*RAB6KIFL KIF2C*

KEGG pathway enrichment of Re-analysis of 22 selected genes

To better understand these 22 selected DEGs' possible pathway, we re-analyzed KEGG pathway enrichment via DAVID (P < 0.05). We found that five core genes (BUB1, MAD2L1, CCNA2, CCNB1, and BIRC5) markedly enriched in the progesterone-mediated oocyte maturation and cell cycle pathway (P = 1.9E-3, Table 6). Among all these genes, the overexpression of BIRC5, TRIP13 and UBE2C was negative correlated with the poor out comes of gastric cancer patients, which was further investigated.

Upregulation of BIRC5, TRIP13 and UBE2C in gastric cancer tissues from clinical patients and are related to cell proliferation

To validate the expression level of these potential genes in cancer tissues, we collected 15 pairs of tumor tissues and adjacent normal tissues of gastric cancer. With RT-PCR assay, we found that BIRC5, TRIP13 and UBE2C were significantly upregulated in tumor tissues (Fig. 6A, B, C), these data showed that these genes may be applied as biomarkers for gastric cancer. To investigate whether these genes exert key functions in cancer, we transfected specific shRNAs into gastric cancer cells. Through CCK-8 assay, we observed that cell

proliferation was significantly suppressed in BIRC5, TRIP13 or UBE2C knockdown cells, respectively (Fig. 6D, E, F).

Knockdown of BIRC5, TRIP13 or UBE2C reduced cisplatin resistance

Cisplatin is one of the most frequent drugs currently used for treating gastric cancer. However, cisplatin resistance is a big obstacle for successfully therapy. We ought to investigate whether these critical genes are related to cisplatin resistance. Then cells with knockdown of each specific gene were treated with cisplatin. Then cell survival fraction and apoptosis were examined. Our data showed that knockdown of BIRC5, TRIP13 or UBE2C significantly inhibited the cell survival fraction after cisplatin treatments (Fig. 7A, B, C). Knockdown of these genes also significantly increased cisplatin-induced cell apoptosis (Fig. 7D, E, F). These data suggested that the high regulation of BIRC5, TRIP13 or UBE2C genes are closely related to cell proliferation as well as cisplatin resistace, which could be used as potential target for treating gastric cancers.

Discussion

In present research, in order to find more core prognostic biomarkers in GC cancer, we used bioinformatical methods based on three profile datasets (GSE13911, GSE66229, and GSE 79973). Statistics suggested that 348 gastric cancer specimens and 141 normal specimens were involved in this study. We uncovered 251 total amount changed DEGs (settings: |logFC| > 0.5 and adjust P value < 0.05) including 64 up-regulated (Log FC > 0) and 187 down-regulated DEGs (Log FC < 0) through GEO2R and Venn software platform. Then, Results from the present study demonstrated 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 nuclear chromosome segregation, sister chromatid segregation, cell division, cell cycle process, mitotic cell cycle process and down-regulated DEGs in organic acid catabolic process, carboxylic acid catabolic process, small molecule catabolic process, lipid modification, fatty acid catabolic process, fatty acid oxidation; 2) for molecular function (MF), up-regulated DEGs were enriched in identical protein binding, microtubule binding, enzyme binding, tubulin binding, cytoskeletal protein binding protein kinase binding and down-regulated DEGs in cofactor binding, coenzyme binding, potassium channel regulator activity, ligand-gated ion channel activity, ligand-gated channel activity; 3) for changes in the cell components (CC), up-regulated DEGs were enriched in condensed chromosome kinetochore, condensed chromosome, centromeric region kinetochore, condensed chromosome, chromosome, centromeric region, midbody and down-regulated DEGs in mitochondrial matrix, mitochondrial part, mitochondrion, intrinsic component of plasma membrane, integral component of plasma Membrane, cytoplasmic vesicle membrane.

BUB1, which is from BUB (budding uninhibited by Benzimidazole) gene families, can encode proteins form a large kinetochore-associated multi-protein complex. It has been suggested that altering BUB1 gene expression levels could disrupt its function, leading to aberrant chromosomal segregation. David Stahl et[15] indicated tumors with low frequency of BUB1 expression were associated with larger tumor size, higher incidence of lymph node metastases, distant metastases and higher UICC stage. Moreover, BUB1 expression was inversely correlated with the residual tumor stage. It was reported that the expression of BUB1 was also associated with taxane sensitivity in other types of cancer as: breast and esophageal cancer[16, 17]. Furthermore, Eisuke Kawakubo et[18] found that BUB1 insufficiency leads to the lack of eNOS expression

both in vitro and in vivo. They also revealed that lack of eNOS expression in gastric cancer tissues with low BUBR1 expression.

MAD2, Mitotic arrest deficient 2, is a key component of the mitotic spindle checkpoint pathway. It plays a vital role in maintaining spindle checkpoint function by generating special signal for monitoring the localization and segregation of chromosomes. Wang et[19]also reported that the reduced expression of the MAD2 resulted in deficient mitotic checkpoint in several human cancers. Du Y et[20] found that Mad2 interference could inhibit anticancer drug-induced apoptosis by up-regulating Bcl-2 and interfering with the mitochondria apoptosis pathway. J Bargiela-I et [21]demonstrated that depletion of Mad2 can delay Mcl-1 degradation, suggesting that exit to mitosis is delayed.

CCNB1 and CCNA2, which regulates cell progression via the G2/M transition during the cell cycle and encoded by related cyclin key genes, play significant roles in the G2/M phase. High level of CCNA2[22] was detected over-expression in various cancers, including GC, non-small cell lung cancer and esophageal squamous cell carcinoma, resulting in uncontrolled cell growth. Up-regulation of CCNB1 expression was confirmed can promote the cell cycle in the G2/M phase, which was in line with the poor survival in GC patients[23]. Moreover, Holloway et[24] indicated CCNB1 arises in the cytoplasm of cells in S-phase, and then it is transported to the nucleus at the G2/M transition where it is broken down during anaphase via a ubiquitin-dependent pathway. A recent study[25] showed that up-regulation of CCNA2 could be an indicator for pituitary adenomas invasiveness, for it played an indispensable role in monitoring protein in the regulation of cell cycle. Paula Müssnich et[26] validated that downregulation of CCNA2 with protein and mRNA levels could decrease cell proliferation.

BIRC5, Baculoviral inhibitor of apoptosis repeat-containing 5, which was well known as the encoder of survivin, a member of inhibitor-of-apoptosis proteins (IAPs) family. During G2/M phase in tumor proliferating cells, BIRC5 plays a key role in cell division located at 17q25, regulating cell apoptosis and process of mitosis. Several reports revealed that BIRC5 not only interacts with caspases but also enables the formation of the chromosomal passenger complex (CPC), which is the vital matter promoting mitosis[27–29]. Nabilsi et al. indicated BIRC5 is related to sex hormones, and it was for the first time making a point that the overexpression of BIRC5 combined to high female sex hormones[30]. Leung et al. reported that low expression of surviving may lead to the decrease of cell cycle functional, which may results in the loss of related cells[31].

Based on patient survival analysis, we focused on the three critical genes, including BIRC5, TRIP13 or UBE2C. It was reported that these three genes were proved to play important roles in progress of various cancers, while after we searched in website about these genes related of GC cancer, we found just few reports have been suggested the correlation. We also performed cell experiments and found that knockdown these genes inhibited cell proliferation, reduced cellular resistance to cisplatin. Our findings provide novel targets for the treatment of gastric cancer.

In conclusion, exploring the Molecular Mechanism of Gastric cancer via Bioinformatics Analysis shows three DEGs (BIRC5, TRIP13 or UBE2C) play key roles in the progression of GC. Hub genes and key pathways of gastric cancer identified using bioinformatics leading to a molecular link between the pathway and gene

expression potentially involved in GC. The current paper associations with bioinformatics analysis identified related genes and cellular pathways involved in GC's emergence and development. However, certain limitations still existed by the lack of cell experimental. Hence, further experimental studies should be put on the agenda to confirm the expression and function of the identified genes at the molecule level, which will help to elucidate GC pathogenesis and identify novel biomarkers or drug targets for improved improvement diagnostics and therapeutics for GC.

Declarations

Conflicts of interest

The authors have no conflicts of interest to disclose.

Acknowledgements

We are grateful to Dr. Qingqiang Xu from Naval Medical University for kind disscussions.

Funding Info

Foundation of Shanghai Municipal Commission of Health and Family Planning NO. 202140074

Ethics statement

All the experiments were approved by the Ethics Committee of The Dahua Hospital and followed the instructions for the Care and Use of Laboratory Animals published by the NIH (Publication No. 96-01).

Consent to participate and for publication

All the authors are consent and approved the submission and publication of this paper.

Authors' contributions

K.Yu. Y.X. and K.Mei.: study concept and design, Data mining and bioinformatic analysis, preparation of manuscript, obtain funding. H.Qian, X.Pan: carried out experiments, data analysis, figures preparation. G.Wang, X. Pan: carried out analysis. K.Mei, D.Zhang: study design, obtained funding.

Availability of data and material

All the data and materials are available for all readers upon request.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-386.

2. Huang L, Hu C, Cao H, Wu X, Wang R, Lu H, Li H and Chen H. MicroRNA-29c Increases the Chemosensitivity of Pancreatic Cancer Cells by Inhibiting USP22 Mediated Autophagy. Cell Physiol Biochem 2018; 47: 747-758.
3. Chen F and Hu SJ. Effect of microRNA-34a in cell cycle, differentiation, and apoptosis: a review. J Biochem Mol Toxicol 2012; 26: 79-86.
4. Kim TH and Dekker J. ChIP-chip. Cold Spring Harb Protoc 2018; 2018:
5. Cives M, Ciavarella S, Rizzo FM, De Matteo M, Dammacco F and Silvestris F. Bendamustine overcomes resistance to melphalan in myeloma cell lines by inducing cell death through mitotic catastrophe. Cell Signal 2013; 25: 1108-1117.
6. Davis S and Meltzer PS. GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor. Bioinformatics 2007; 23: 1846-1847.
7. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM and Sherlock G. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000; 25: 25-29.
8. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H and Kanehisa M. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 1999; 27: 29-34.
9. Huang da W, Sherman BT and Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.
10. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ and von Mering C. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2015; 43: D447-452.
11. Krispel CM, Chen CK, Simon MI and Burns ME. Prolonged photoresponses and defective adaptation in rods of Gbeta5-/- mice. J Neurosci 2003; 23: 6965-6971.
12. Szasz AM, Lanczky A, Nagy A, Forster S, Hark K, Green JE, Boussioutas A, Busuttil R, Szabo A and Gyorffy B. Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients. Oncotarget 2016; 7: 49322-49333.
13. Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98-W102.
14. Fisher RA. On the Interpretation of χ² from Contingency Tables, and the Calculation of P. Journal of the Royal Statistical Society 1922; 85: 87-94.
15. Stahl D, Braun M, Gentles AJ, Lingohr P, Walter A, Kristiansen G and Gutgemann I. Low BUB1 expression is an adverse prognostic marker in gastric adenocarcinoma. Oncotarget 2017; 8: 76329-76339.
16. McGrogan BT, Gilmartin B, Carney DN and McCann A. Taxanes, microtubules and chemoresistant breast cancer. Biochim Biophys Acta 2008; 1785: 96-132.
17. Tanaka K, Mohri Y, Ohi M, Yokoe T, Koike Y, Morimoto Y, Miki C, Tonouchi H and Kusunoki M. Mitotic checkpoint genes, hsMAD2 and BubR1, in oesophageal squamous cancer cells and their association with 5-fluorouracil and cisplatin-based radiochemotherapy. Clin Oncol (R Coll Radiol) 2008; 20: 639-646.

18. Kawakubo E, Matsumoto T, Yoshiya K, Yamashita S, Jogo T, Saeki H, Oki E, Furuyama T, Oda Y and Maehara Y. BUBR1 Insufficiency Is Correlated with eNOS Reduction Experimentally In Vitro and In Vivo, and in Gastric Cancer Tissue. Anticancer Res 2018; 38: 6099-6106.
19. Wang X, Jin DY, Ng RW, Feng H, Wong YC, Cheung AL and Tsao SW. Significance of MAD2 expression to mitotic checkpoint control in ovarian cancer cells. Cancer Res 2002; 62: 1662-1668.
20. Du Y, Yin F, Liu C, Hu S, Wang J, Xie H, Hong L and Fan D. Depression of MAD2 inhibits apoptosis of gastric cancer cells by upregulating Bcl-2 and interfering mitochondrion pathway. Biochem Biophys Res Commun 2006; 345: 1092-1098.
21. Bargiela-Iparraguirre J, Prado-Marchal L, Pajuelo-Lozano N, Jimenez B, Perona R and Sanchez-Perez I. Mad2 and BubR1 modulates tumourigenesis and paclitaxel response in MKN45 gastric cancer cells. Cell Cycle 2014; 13: 3590-3601.
22. Yasuda M, Takesue F, Inutsuka S, Honda M, Nozoe T and Korenaga D. Overexpression of cyclin B1 in gastric cancer and its clinicopathological significance: an immunohistological study. J Cancer Res Clin Oncol 2002; 128: 412-416.
23. Shi Q, Wang W, Jia Z, Chen P, Ma K and Zhou C. ISL1, a novel regulator of CCNB1, CCNB2 and c-MYC genes, promotes gastric cancer cell proliferation and tumor growth. Oncotarget 2016; 7: 36489-36500.
24. Roncalli M, Bosari S, Marchetti A, Buttitta F, Bossi P, Graziani D, Peracchia A, Bonavina L, Viale G and Coggi G. Cell cycle-related gene abnormalities and product expression in esophageal carcinoma. Lab Invest 1998; 78: 1049-1057.
25. Zhao P, Zhang P, Hu W, Wang H, Yu G, Wang Z, Li C, Bai J and Zhang Y. Upregulation of cyclin B1 plays potential roles in the invasiveness of pituitary adenomas. J Clin Neurosci 2017; 43: 267-273.
26. Mussnich P, Raverot G, Jaffrain-Rea ML, Fraggetta F, Wierinckx A, Trouillas J, Fusco A and D'Angelo D. Downregulation of miR-410 targeting the cyclin B1 gene plays a role in pituitary gonadotroph tumors. Cell Cycle 2015; 14: 2590-2597.
27. Shin S, Sung BJ, Cho YS, Kim HJ, Ha NC, Hwang JI, Chung CW, Jung YK and Oh BH. An anti-apoptotic protein human survivin is a direct inhibitor of caspase-3 and -7. Biochemistry 2001; 40: 1117-1123.
28. Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T and Reed JC. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res 1998; 58: 5315-5320.
29. Rosa J, Canovas P, Islam A, Altieri DC and Doxsey SJ. Survivin modulates microtubule dynamics and nucleation throughout the cell cycle. Mol Biol Cell 2006; 17: 1483-1493.
30. Nabilsi NH, Broaddus RR, McCampbell AS, Lu KH, Lynch HT, Chen LM and Loose DS. Sex hormone regulation of survivin gene expression. J Endocrinol 2010; 207: 237-243.
31. Leung CG, Xu Y, Mularski B, Liu H, Gurbuxani S and Crispino JD. Requirements for survivin in terminal differentiation of erythroid cells and maintenance of hematopoietic stem and progenitor cells. J Exp Med 2007; 204: 1603-1611.

Supplemental Figures S1-s2

Supplemental Figures S1-S2 are not available with this version.

Figures

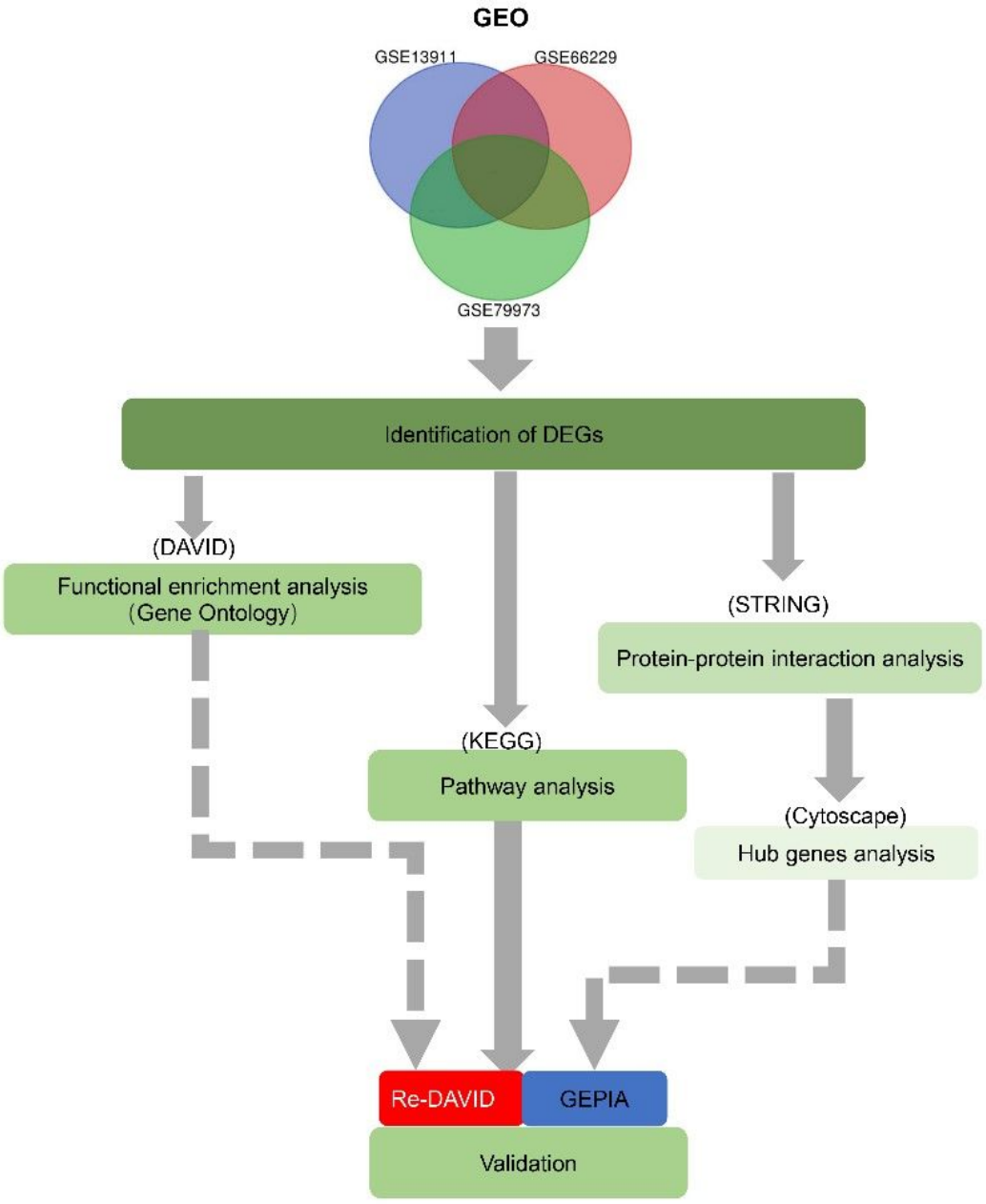

Figure 1

The flow diagram of this study.

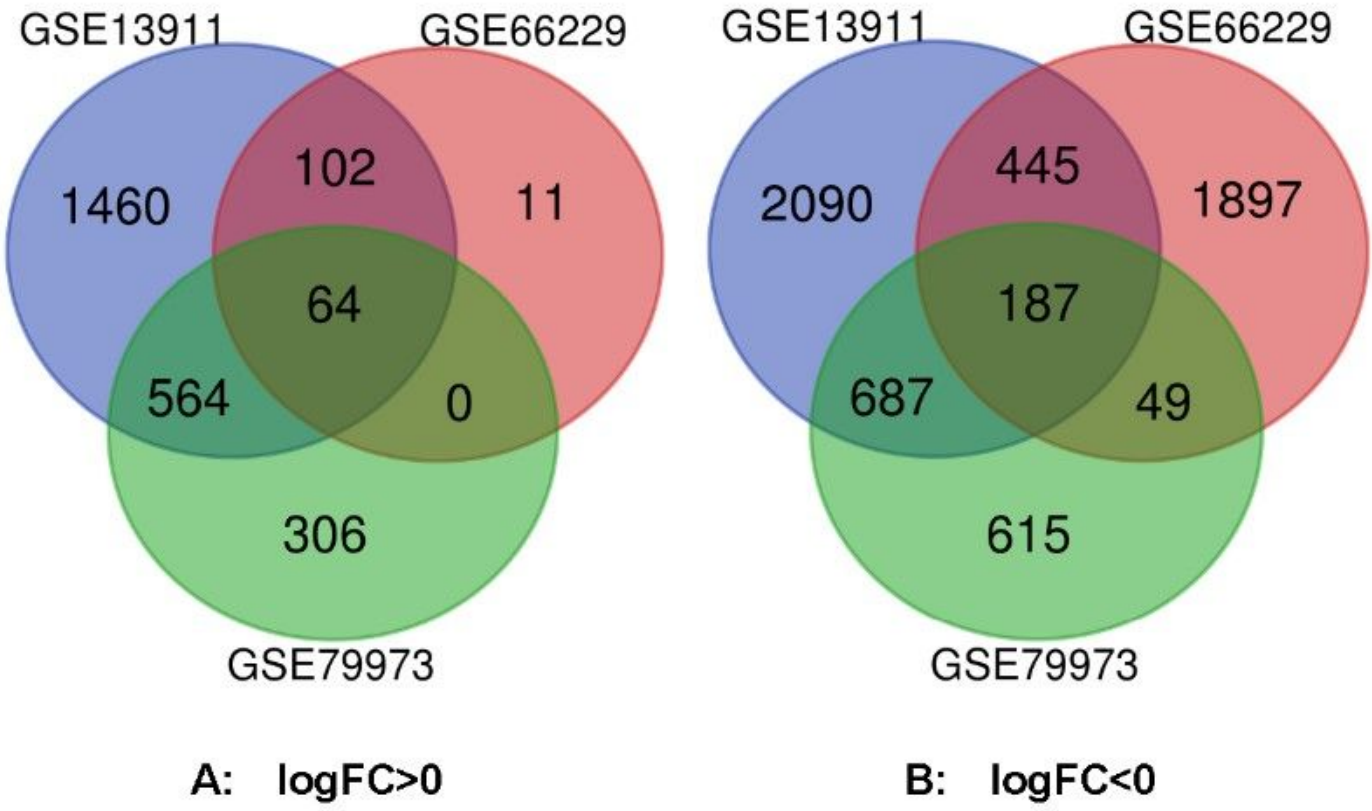

Figure 2

Authentication of 251 common DEGs in the three datasets (GSE13911, GSE66229, and GSE 79973) through Venn Diagrams software (available online: http://bioinformatics.psb.ugent.be/webtools/Venn/). Different color represents different datasets. A: 64 DEGs were upregulated in the three datasets (logFC>0). B: 187 DEGs were downregulated in three datasets (logFC<0).

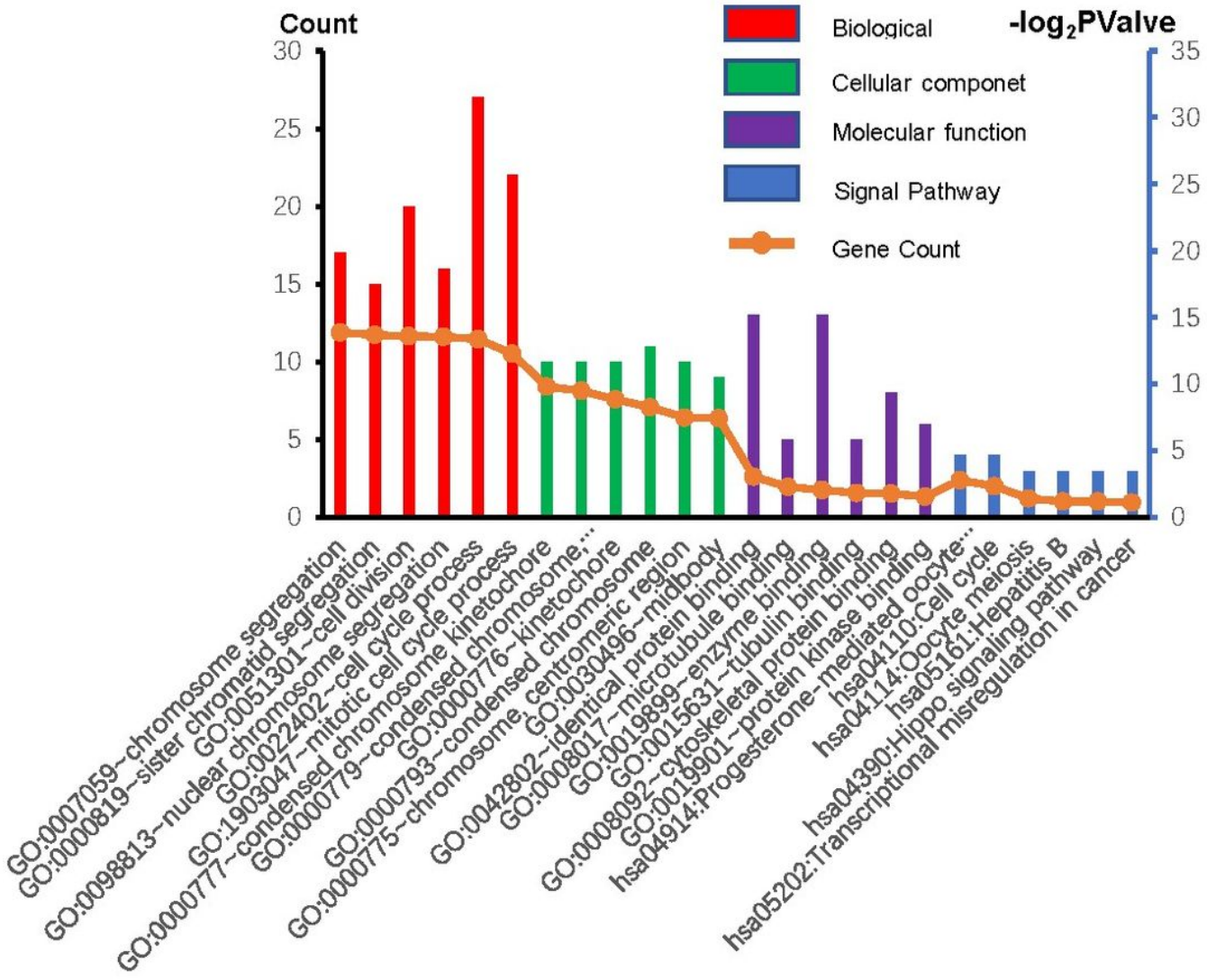

Figure 3

GO terms and KEGG pathways of DEGs significantly enriched in GC. DEGs: differentially expressed genes; GO: gene ontology; GC: gastric cancer; KEGG: Kyoto Encyclopedia of Genes and Genomes.

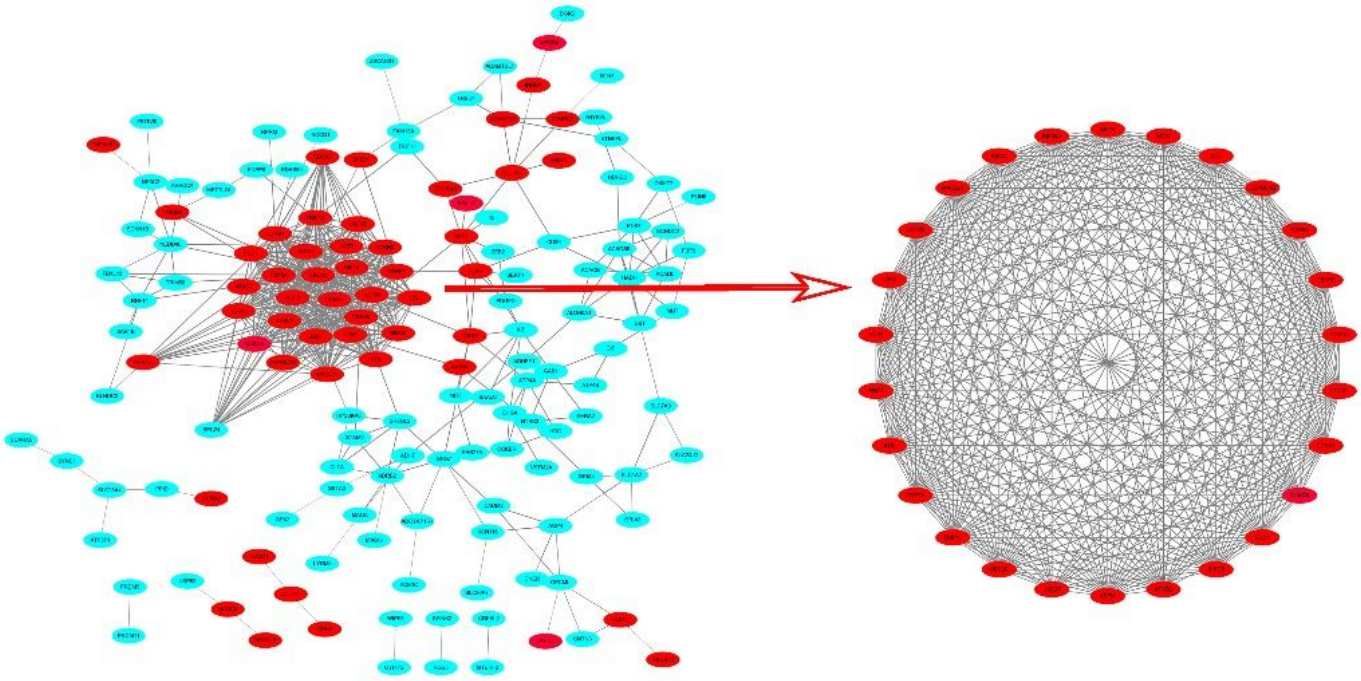

Figure 4

Common DEGs PPI network constructed by STRING online database and Module analysis. A There were a total of 251 DEGs in the DEGs PPI network complex. The nodes meant proteins; the edges meant the interaction of proteins; green circles meant down-regulated DEGs and red circles meant up-regulated DEGs. B Module analysis via Cytoscape software.

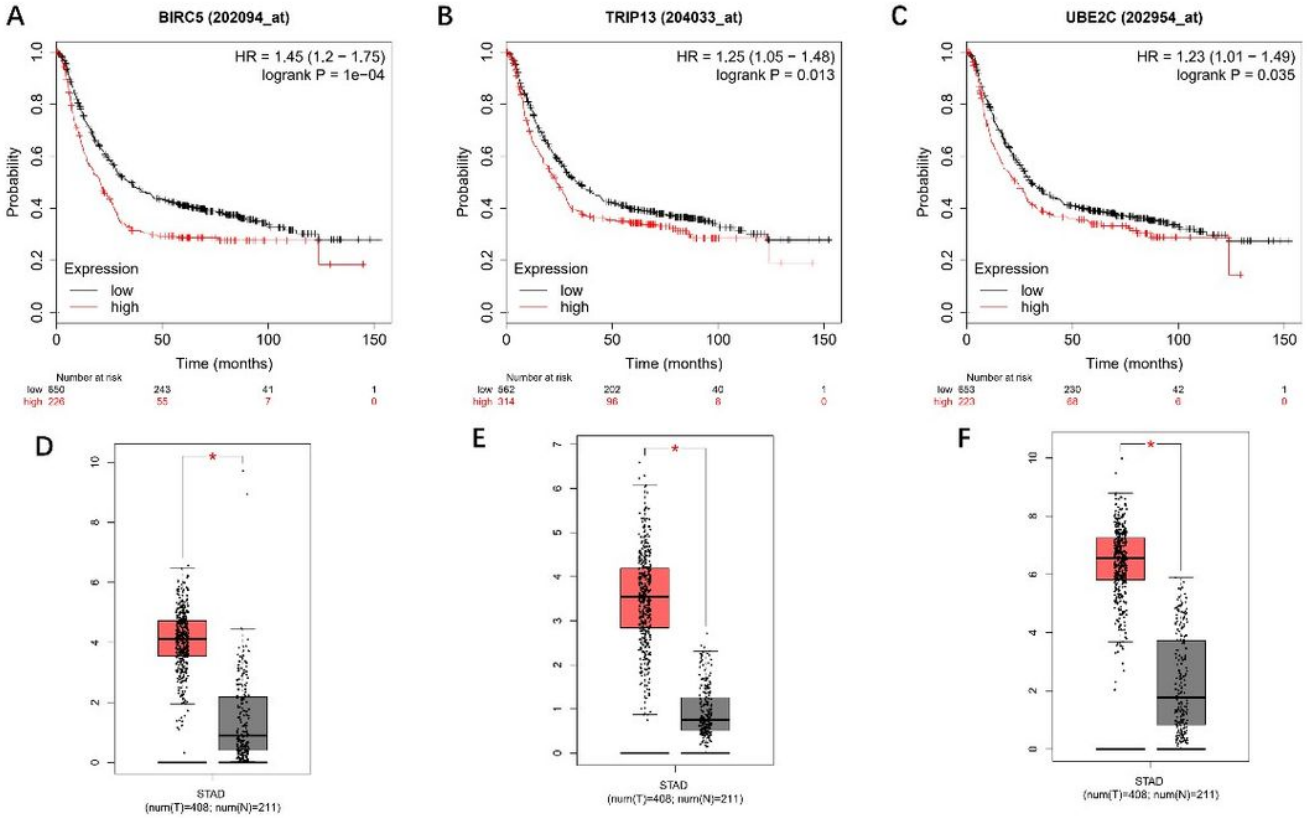

Figure 5

Analysis of BIRC5, TRIP13 and UBE2C genes in patient tissues and the correlation with survival. A-C: overall survival of gastric cancer patients with high expression and low expression of BIRC5 (A), TRIP13 (B) and UBE2C (C) genes. D-E: expression of these genes were analyzed with Gepia tools.

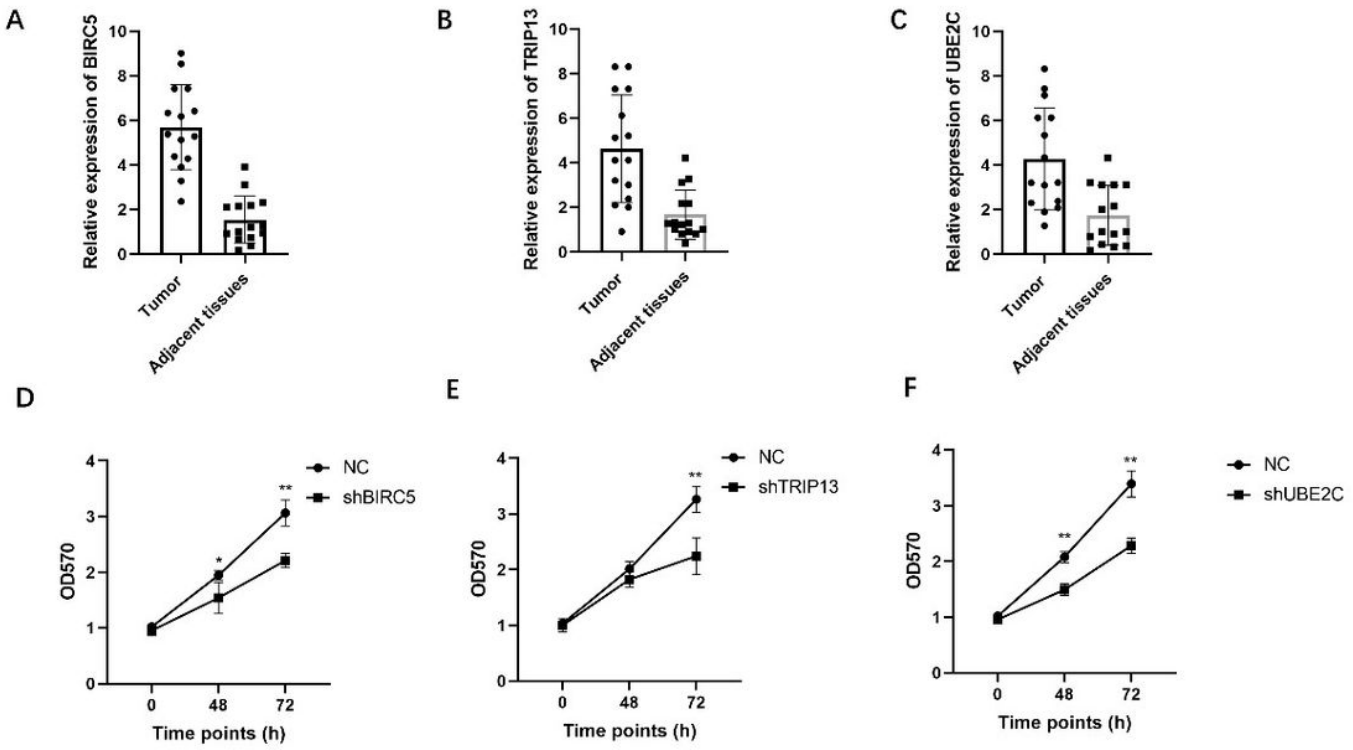

Figure 6

Increased level of BIRC5, TRIP13 and UBE2C genes are related to cell proliferation of gastric cancer cells. A-C: expression of BIRC5, TRIP13 and UBE2C genes in 15 pairs of tumor and the adjacent normal tissues. D-E: knockdown of either BIRC5, TRIP13 or UBE2C genes significantly suppressed cancer cell proliferation.

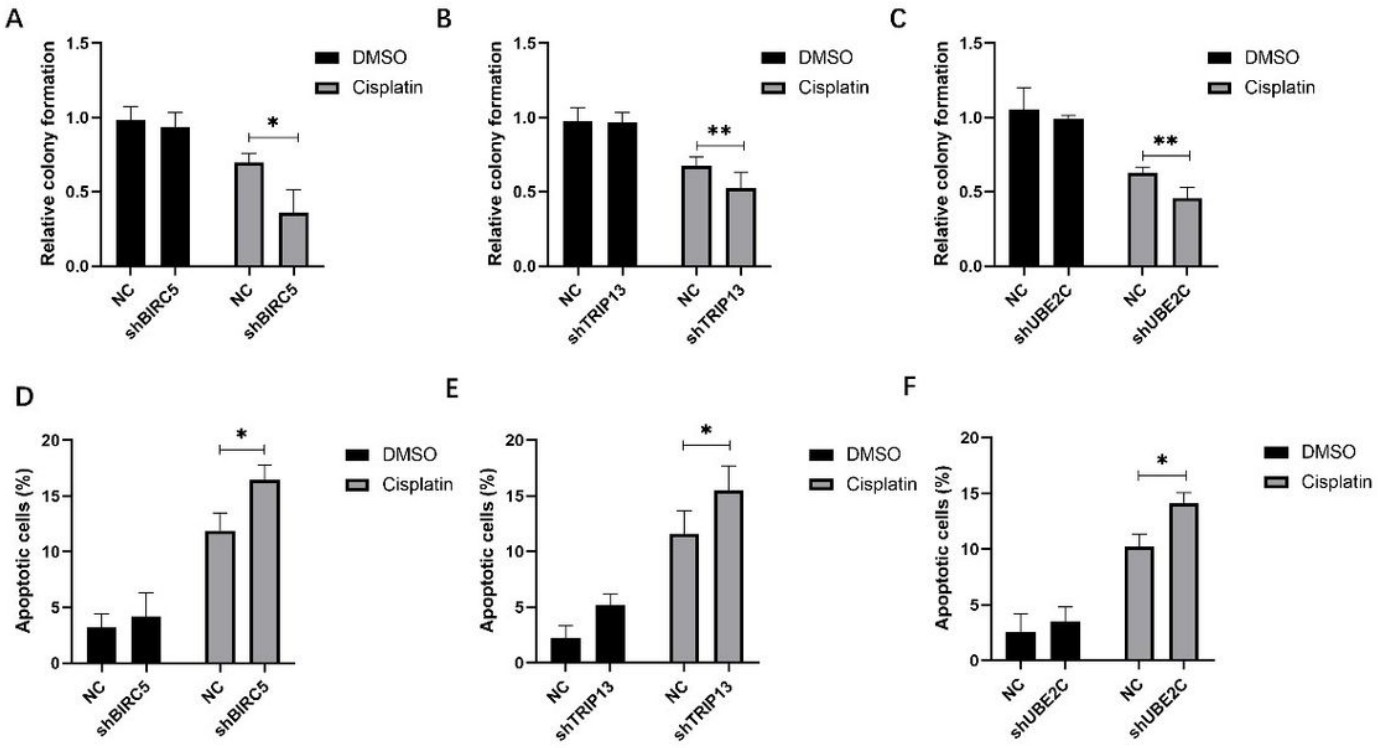

Figure 7

Increased level of BIRC5, TRIP13 and UBE2C genes confers cellular resistance to cisplatin in GC cells. A-C: colony formation assay in cells with shRNA transfected targeting BIRC5, TRIP13 or UBE2C genes. D-F: cell apoptosis was detected in BIRC5, TRIP13 and UBE2C knockdown cells after treated with cisplatin.