

# Expression and Prognosis Analyses of Insulin-Like Growth Factor 2 mRNA Binding Protein Family in Human Pancreatic Cancer

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## Research article

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# Abstract

**Background:** Despite advances in early diagnosis and treatment, cancer remains the leading cause of mortality worldwide. Insulin-like Growth Factor 2 mRNA Binding Protein (IGF2BP) family had been reported to be involved in a variety of human malignant tumors. However, little was known about their expression and prognostic value in human pancreatic cancer. So, we performed a detailed cancer versus normal differential analysis.

**Methods:** The Cancer Genome Atlas (TCGA) and Gene Expression Profiles Interactive Analysis (GEPIA) databases were performed to analyze the mRNA expression levels of the IGF2BP family in various cancers, including pancreatic cancer. Then, the LinkedOmics and GEPIA databases were used to assess the relation between the expression levels of IGF2BPs and overall survival (OS). Then, univariate and multivariate COX regression analysis were established and subgroup of Grade&Stage were analyzed. The signaling pathway associated with IGF2BP2 and IGF2BP3 were then investigated via gene set enrichment analysis.

**Results:** IGF2BP2 and IGF2BP3 were found to be associated with each subset of OS and Grade&Stage. Further clinical correlation analysis of IGF2BP2 and IGF2BP3 confirmed that IGF2BP2 and IGF2BP3 were fundamental factors in promoting pancreatic cancer progression.

**Conclusion:** IGF2BP2 and IGF2BP3 were key factors in promoting the progression of pancreatic cancer and was closely related to overall survival.

## Introduction

Pancreatic cancer is a high-mortality tumor with a five-year overall survival rate of about 7% [1, 2]. Among cancer-related death causes, this malignant tumor ranks fourth in the United States and sixth in China [1, 3]. Approximately 80% of patients with pancreatic cancer have dissemination at the time of diagnosis [1, 4]. These patients have lost the chance of radical treatment of pancreatic cancer. In the past decade, despite the advance of anti-metabolism therapy and targeted therapy, the overall survival rate of patients has not been significantly improved due to the late pathological stage, high invasive phenotype and chemotherapy resistance.

Insulin-like growth factor 2-mRNA binding proteins (IGF2BPs), also known as IGF-II mRNA-binding proteins (IMP), were encoded by different genes that belong to the regulatory RNA binding protein family and were involved in the localization of their target RNA, stability and translation control [5]. As the names of these proteins indicate, they are recognized members of the IGF axis that can be linked to IGF2 transcripts [6, 7]. To date, insulin-like growth factor 2 mRNA binding proteins, including IGF2BP1 (IMP1), IGF2BP2 (IMP2), and IGF2BP3 (IMP3), are a unique family of m6A readers that target the common m6A sequence by recognition thousands of mRNA transcripts [8]. In mammals, the protein domains of the three members of the IGF2BP protein family are strikingly similar. All three members of the protein family contain two N-terminal RRM and four C-terminal hnRNPK homology (KH) domains. The latter is

arranged in two dual domains (KH1 + 2 and KH3 + 4) [9]. Consistent with the conservation of six potential RNA-binding domains, all three IGF2BPs bind to single-stranded RNA in vitro and in vivo [9–11]. However, the role of the entire IGF2BPs family in pancreatic cancer remained controversial. Therefore, it was necessary to probe the role of the IGF2BPs family in the pancreatic cancer.

The Cancer Genome Atlas (TCGA) is considered to be the largest cancer database, containing more than 20,000 primary cancer samples and normal matching samples for multiple cancer types. Therefore, we can use bioinformatics methods to study tumor gene data more deeply. To evaluate the relationship between the IGF2BP family and pancreatic cancer progression, our study analyzed the mRNA expression of pancreatic cancer in TCGA by R software and verified it in clinical patients.

## Materials And Methods

### *GEPIA Datasets*

The Gene Expression Profiling Interactive Analysis (GEPIA) is a newly web-based tool for gene expression analysis between the tumor and normal data from the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx), applying a standard processing pipeline. It provides customizable functions such as tumor and normal differential expression analysis, and we could demonstrate the expression of IGF2BP1-3 in pancreatic cancer and normal tissue. GEPIA possesses key variable and interactive functions, including profiling plotting, differential expression analysis, patient survival analysis, similar gene detection and dimensionality reduction analysis.

### *LinkedOmics Dataset*

LinkedOmics is a new and unique tool in the software ecosystem for disseminating data from all 32 TCGA cancer types. It can be used to access, analyze, and compare multiomics data within and across tumor types. We performed a prognosis analysis for the IGF2BP gene family using the LinkedOmics pancreatic cancer dataset.

### *TCGA data acquisition and differentially expressed IGF2BP gene analysis*

The pancreatic cancer data in TCGA contained 178 pancreatic cancer cases with significant information, including pathological grade and clinical stage. All mRNA expression data were downloaded and further analyzed by R software along with clinical data.

We used the R software “Limma” package to normalize the original expression levels of mRNAs downloaded from TCGA. “Limma” package was then further utilized to analyze the expression of each IGF2BP gene between every grade and stage of cancer tissues, setting P-value <0.05 as the filter condition for differentially expressed IGF2BP.

### *Gene set enrichment analysis of pancreatic cancer*

Gene enrichment analysis (GSEA) (version 3.0, the broad institute of MIT and Harvard, <http://software.broadinstitute.org/gsea/downloads.jsp>) was conducted between pancreatic cancer and normal tissues to study the biological characteristics of pancreatic cancer. In detail, the “collapse data set to gene symbols” was set to false, the number of marks was set to 1000, the “permutation type” was set to phenotype, the “enrichment statistic” was set to weighted, and the Signal2Noise metric was used for ranking genes. High expression group was used as experimental group and low expression group was used as reference group. “c2.cp.kegg.v7.0.symbols.gmt” gene sets database was used for enrichment analysis. Gene set size >500 and <15, FDR <0.25, and nominal P-value <0.05 were regarded as the cut-off criteria.

### ***Functional enrichment analyses of pancreatic cancer***

Tools were utilized to conduct the IGF2BP2 and IGF2BP3 functional enrichment analyses including Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO). Database for Annotation, Visualization, and Integrated Discovery (DAVID, <https://david.ncifcrf.gov/>) were applied to identify enriched KEGG and GO themes.

### ***Cell Lines and regents***

The human pancreatic cancer cell line ASPC-1, BxPC-3, PANC-1, MIA Paca-2 and HPDE6-C7 were purchased from the University of Colorado Cancer Center Cell Bank and cultured in RPMI 1640 and DMEM medium supplemented with 10% FBS (Invitrogen, Carlsbad, CA, USA) at 37°C in a 5% CO<sub>2</sub> atmosphere, respectively. Cells were digested and passaged when cell fusion reached 80-90%.

### ***Quantitative reverse transcription-polymerase chain reaction***

Total RNA was extracted from the ASPC-1, BxPC-3, PANC-1, MIA Paca-2 and HPDE6-C7 cell lines using the TRIzol reagent (Life Technologies) according to the instructions provided by the manufacturer. Total RNA (1 µg) was used as template to synthesize complementary DNA (cDNA) using a PrimeScript RT Reagent Kit with cDNA Eraser (Takara Biotechnology). Subsequently, qRT-PCR was performed using the SYBR Premix Ex Taq (Takara Bio Inc). The primer sequences used in real-time PCR were listed in Table S1. All qRT-PCR assays were performed on an ABI 7900 system (Applied Biosystems).

### ***Cell proliferation assay***

The Cell Counting Kit-8 (CCK-8) assay (MedChemExpress) was used according to the protocol provided by the manufacturer to assess cell proliferation. ASPC-1 and SW1990 cells were seeded in 96-well plates ( $5 \times 10^3$  cells/plate) and cultured at 37°C. 10ul CCK8 solution was given to each well of the plate after every incubation times: 0h, 24h, 48h, 72h and 96h. The optical density (OD) was measured, at 1-4 days, at a wavelength of 450 nm using a Multiskan FC (ThermoFisher Scientific, Inc).

### ***Colony formation assay***

ASPC-1 and SW1990 cell lines were seeded in 6-well plates ( $1 \times 10^3$  cells/plate), cultured for 14 days. Then, cells fixed with 10% formaldehyde for 5 minutes and stained with 1% crystal violet for 30 s prior to counting the number of colonies.

### ***Cell invasion assay***

Cell invasion was analyzed with transwell plates (24-well insert, 8  $\mu$ m pore size; BD Biosciences, Bedford, MA, USA). The filters (Corning Inc., USA) were coated with 55  $\mu$ L Matrigel (1:8 dilution; BD Biosciences). The  $5 \times 10^4$  cells were suspended in 100  $\mu$ L RPMI-1640 and DMEM medium without serum and seeded in the upper chamber. Next, 600  $\mu$ L 90% RPMI-1640 and DMEM supplement with 10% FBS was added to the bottom chamber. After incubation for 24h, the chambers were fixed by 4% paraformaldehyde for 30 min and then stained by 0.1% crystal violet for 30 minutes. At last, we used a magnification microscope to count the amount of the invasion cells in the bottom of the chamber.

### ***Statistical analysis***

The experiments in this article were performed in triplicate, and the data were expressed as means  $\pm$  standard deviation. A t-test was utilized for the statistical analysis of the data from 2 groups. The comparisons of multiple groups were performed with one-way ANOVA followed by an LSD-t test.  $P < 0.05$  was considered to be significant.

## **Results**

### ***Transcriptional Levels of IGF2BPs in Patients with Pancreatic Cancer***

Three IGF2BPs factors were identified in mammalian cells, and the expression levels of IGF2BPs were compared in various cancers via the GEPIA database. IGF2BP1 mRNA expression level was not up-regulated in pancreatic cancer, but IGF2BP2 and IGF2BP3 were up-regulated in pancreatic cancer to varying degrees (Figure. 1).

GEPIA database was utilized to further analyze whether there was a difference in the expression of IGF2BPs factor between pancreatic cancer and normal pancreatic tissue. In studies of ONCOMINE, the expression of IGF2BP1 in pancreatic cancer tissue was not significantly different from that of normal pancreatic tissue. However, in Segara and Pei Pancreas' dataset, IGF2BP2 is over-expressed compared with that in the normal sample in pancreatic carcinoma with a fold change of 3.446 and 2.657, respectively (Table 1) [12-16]. Regard to IGF2BP3, four pancreas' dataset all indicated an overexpression in both pancreatic carcinoma and pancreatic ductal adenocarcinoma (Table 1).

### ***Relationship between the mRNA Levels of IGF2BPs and the Clinicopathological Parameters of Patients with Pancreatic Cancer.***

We utilized the TCGA database to compare the expression levels of the IGF2BP family in normal pancreatic tissues and pancreatic cancer. Among them, the expression levels of IGF2BP2 and IGF2BP3

were significantly increased in pancreatic cancer (Figure. 2A). With the GEPIA (Gene Expression Profiling Interactive Analysis) dataset (<http://gepia.cancer-pku.cn/>), we compared the mRNA expression level of IGF2BP protein family between pancreatic cancer and normal tissue. The results showed that the expression of IGF2BP1 genes in pancreatic cancer was not different from that in normal pancreatic tissue samples. However, a higher expression levels of IGF2BP2 and IGF2BP3 were observed (Figure. 2B).

### ***Clinical correlation analysis in Pancreatic Cancer Patients***

Further, we performed a prognostic analysis of IGF2BP1, IGF2BP2, and IGF2BP3 in pancreatic cancer with the LinkedOmics and GEPIA dataset. In the LinkedOmics dataset, the high expression of IGF2BP1, IGF2BP2, and IGF2BP3 was associated with substantially poor overall survival (OS) of pancreatic cancer (Figure. 3A). Interestingly, for IGF2BP1 and IGF2BP2, consistent results were observed in the prognostic analysis of the GEPIA dataset (Figure. 3B).

The association between IGF2BP1-3 and each subset of grade&stage was analyzed by R software via the Wilcox test. P-value<0.05 was considered to be statistically significant. We found that the expression level of IGF2BP2 and IGF2BP3 continuously increased in each subgroup of grade, except for the grade 4 (Figure. 4A). In the clinical stage, IGF2BP1-3 gradually increased in the subgroup but there was no statistical difference (Figure. 4B).

### ***Univariate and multivariate COX regression analysis***

Cox's proportional hazards model was applied to analyze related factors that may affect the overall survival of pancreatic cancer patients, in which IGF2BP2 and IGF2BP3 were identified as independent prognostic factors (Figure. 5A, B). In both univariate and multivariate analysis, low expression of IGF2BP2 and 3 suggested better OS. In multivariate analysis, the HR of IGF2BP2 was 1.415 with 95% CI was 1.133–1.768, and the HR of IGF2BP3 was 1.052 with a 95% CI was 1.017–1.019. Further, based on the results of multivariate cox regression analysis, we established a nomogram model that may predict patient's survival (Figure. 5C).

### ***Gene mutation information***

Cbioportol was utilized to calculate the gene mutation information in pancreatic cancer samples from TCGA database. In general, missense mutation was detected to be the most frequent mutation classification in pancreatic cancer. Collectively, SNP and C>T were confirmed to be the most fundamental Variant Type and SNV class, respectively. The median variation for each sample is approximately 26. Finally, we detected the top 10 mutations in pancreatic cancer, including TP53, KRAS, TTN, MUC16, SMAD4, CDKN2A, RYR1, RNF43, PCDH15 and ARID1A (Figure. 6A). Then, compared with the top 10 mutation types, we detected the mutation types of IGF2BP1-3 including missense mutation and silent (Figure. 6B).

### ***Gene set enrichment analysis of IGF2BP2-3***

In order to fully understand the biological attributes of IGF2BP2 and IGF2BP3, we conducted gene set enrichment analysis. Based on the results of GSEA, the top three up-regulated enriched pathway terms of IGF2BP2 were: adherens junction, pentose phosphate pathway and pentose and glucuronate interconversions; the principal down-regulated biological pathway enriched of IGF2BP2 were: primary bile acid biosynthesis, neuroactive ligand receptor interaction and glycosphingolipid biosynthesis ganglio series (Figure. 7A). The top three up-regulated enriched pathway terms of IGF2BP3 were: pathogenic Escherichia coli infection, thyroid cancer and adherens junction; the principal down-regulated biological pathway enriched of IGF2BP3 were: glycine serine and threonine metabolism and neuroactive ligand receptor interaction (Figure. 7B).

### ***Gene functional enrichment analysis***

In order to fully understand the biological attributes of IGF2BP2 and IGF2BP3, we utilized the Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) analysis. We biologically enriched genes, based on the results of DAVID, positively related to the expression levels of IGF2BP2 and IGF2BP3. In GO analysis, the three biological processes in which genes positively related to IGF2BP2 expression level are involved: regulation of cytoskeleton organization, neutrophil activation and neutrophil degranulation. The three cell components involved in these co-expressed genes include: adherens junction, cell-substrate junction and focal adhesion. In addition, the three main molecular functions of these co-expressed genes include: cell adhesion molecule binding, cadherin binding and actin binding (Figure. 8A). In pathway analysis of genes that positively related to IGF2BP2 expression level, the top three enriched terms were: salmonella infection, shigellosis and pathogenic escherichia coli infection (Figure. 8A). In GO analysis, the three biological processes in which genes positively related to IGF2BP3 expression level are involved include: viral life cycle, regulation of chromosome organization and regulation of mRNA metabolic process. The three cell components involved in these co-expressed genes include: adherens junction, cell-substrate junction and focal adhesion. In addition, the three main molecular functions of these co-expressed genes include: cell adhesion molecule binding, cadherin binding and transcription coregulator activity (Figure. 8B). In KEGG pathway analysis of genes that positively related to IGF2BP2 expression level, the top three enriched terms were: human papillomavirus infection, endocytosis and salmonella infection (Figure. 8B). Finally, the result of GO and KEGG analysis to biologically enrich genes that were positively correlated with IGF2BP2 and IGF2BP3 expression levels, the top 10 relevant biological processes including: cell junction organization, salmonella infection, mitotic nuclear division, cell cycle, et al. (Supplementment Figure 1).

### ***IGF2BP2 and IGF2BP3 expression and function in cell lines***

For further exploration of IGF2BP2 and IGF2BP3 expression in cell lines, qPCR was performed. As expected, IGF2BP2 and IGF2BP3 protein was significantly increased in pancreatic cancer cell lines compared with HPDE6-C7, meanwhile the expression of IGF2BP2 and IGF2BP3 in pancreatic cancer cell lines showed further increased (Figure 9A). As predicted in GSEA analysis above, we inferred IGF2BP2 and IGF2BP3 promotes proliferation or metastasis of pancreatic cancer to accelerate its progression. The

growth rate of pancreatic cancer cell lines transfected with IGF2BP2 siRNA and IGF2BP3 siRNA was significantly slower than that of NC group (Figure 9B, C). In cell invasion analysis, knockdown of IGF2BP2 and IGF2BP3 significantly decreased the invasion rate of SW1990 (Figure 9D).

## Discussion

In the past few years, despite tremendous efforts in pancreatic cancer research, the 5-year survival rate has not improved significantly. Patients with early pancreatic cancer have a good prognosis and can be cured by surgery combined with adjuvant therapy. However, most patients with advanced pancreatic cancer cannot undergo surgical resection alone. For patients with advanced pancreatic cancer, it was essential to explore more effective prognostic markers and therapeutic targets. Therefore, we screened the IGF2BP protein family through bioinformatics and conducted a differential analysis. IGF2BP2 and IGF2BP3 related to pancreatic cancer progression and survival were further analyzed, and their functions were verified in in vitro experiments.

In the preliminary analysis results, three members of the IGF2BP protein family were identified to have differential expression between pancreatic cancer and adjacent tissues. Further analysis confirmed that only IGF2BP2 and IGF2BP3 were associated with pancreatic cancer progression. Therefore, IGF2BP2 and IGF2BP3 were studied only by gene enrichment analysis to assess their cell compositions, molecular functions and biological characteristics.

In gene set enrichment analysis, base excision repair (BER) pathway has been shown to be the most relevant pathway for IGF2BP2. Notably, BER pathway played a significant role in maintain genomic integrity, many human health issues would occur when any part of BER pathway was aberrant [17]. This pathway started with glycosylation enzymes and recognized and excised lesions through the cleavage of glycosidic bonds [17]. Dianov et al. verified that the aberrant P53 signal pathway could lead to a failure of the BER coordination mechanism, overexpression of APE1 and genomic instability [18]. In our enrichment results, the expression level of P53 signaling pathway was also up-regulated, which was consistent with the conclusion of Dianov et al. Although, the relationship between abnormalities in the BER pathway and the development and prognosis of cancer has been studied [19–21]. In pancreatic cancer, whether IGF2BP2 is associated with this process has not been elucidated. The positive correlation between pathogenic *Escherichia coli* (*E. coli*) infection and colon cancer has been confirmed by multiple studies [22, 23]. The infection of pathogenic *E. coli* destroys the microenvironment of the microflora in the intestinal tract, thereby inducing colon cancer [22, 23, 24]. In studies of pathogenic *Escherichia coli* infection-induced pathways for pancreatic cancer, there was a lack of clear evidence that this pathway was associated with pancreatic cancer. The up-regulation of IGF2BP3 expression in pancreatic cancer tissues provides the possibility to study this pathway. IGF2BP3 imbalance caused pancreatic cancer may be related to pathogenic *E. coli* infection.

The autoimmune response to IGF2BP2 observed in hepatocellular carcinoma, colorectal, ovarian, and breast cancer supports the potential of autoantibodies against IGF2BP2 as biomarkers for cancer

screening, diagnosis, and prognosis [5]. Consistent with the results of our COX regression model in pancreatic cancer, overexpression of IGF2BP2 in basal-like breast cancer and esophageal adenocarcinoma predicts short-term survival for patients. At the cellular level, IGF2BP2 enhances genomic instability and stimulates cancer cell proliferation and migration. In addition, current research suggests that IGF2BP2 is involved in the maintenance of cancer stem cells, suggesting that IGF2BP2 may be important for chemoresistance and relapse of the disease. In addition, IGF2BP3 may differentiate normal tissues from cancerous tissues and serve as a prognostic marker for colorectal, hepatocellular, and ovarian clear-cell carcinomas. Previous research has confirmed IGF2BP3 involved in cell growth and migration in early embryonic development. Nevertheless, the use of IGF2BP2 and IGF2BP3 alone had no effect on tumor growth in mice, and IGF2BP2 and IGF2BP3 was unlikely to have a direct effect on tumors. Similarly, both of our results confirmed the role of IGF2BP2 and IGF2BP3 in inhibiting tumor progression.

## Conclusion

In summary, we have successfully revealed that members of the IGF2BP protein family can be used for diagnosis and prognosis of advanced pancreatic cancer. Both IGF2BP2 and IGF2BP3 have great potential to become biomarkers for pancreatic cancer, which have been verified in clinical patients. Although we have explored the mutation types and possible carcinogenic mechanisms of IGF2BP2 and IGF2BP3 in pancreatic cancer, mechanisms that promote the progression of pancreatic cancer need further study.

## Declarations

### Authors Contributions

CXH and QXH designed the study. ZCF collated the data. CXH and HSY performed the data analyses and produced the initial draft of the manuscript. CXH obtained the results and validated them. All authors had read and approved the final manuscript.

### Competing interests

The authors declare no competing interests.

### Acknowledgements

None.

### Ethics Statement

The study was approved by the Academic Committee of Changzhou Second People's Hospital affiliated to Nanjing Medical University and was conducted in accordance with the principles expressed in the Helsinki Declaration. All datasets are from published literature, so it can be confirmed that all written informed consent has been obtained.

## Funding

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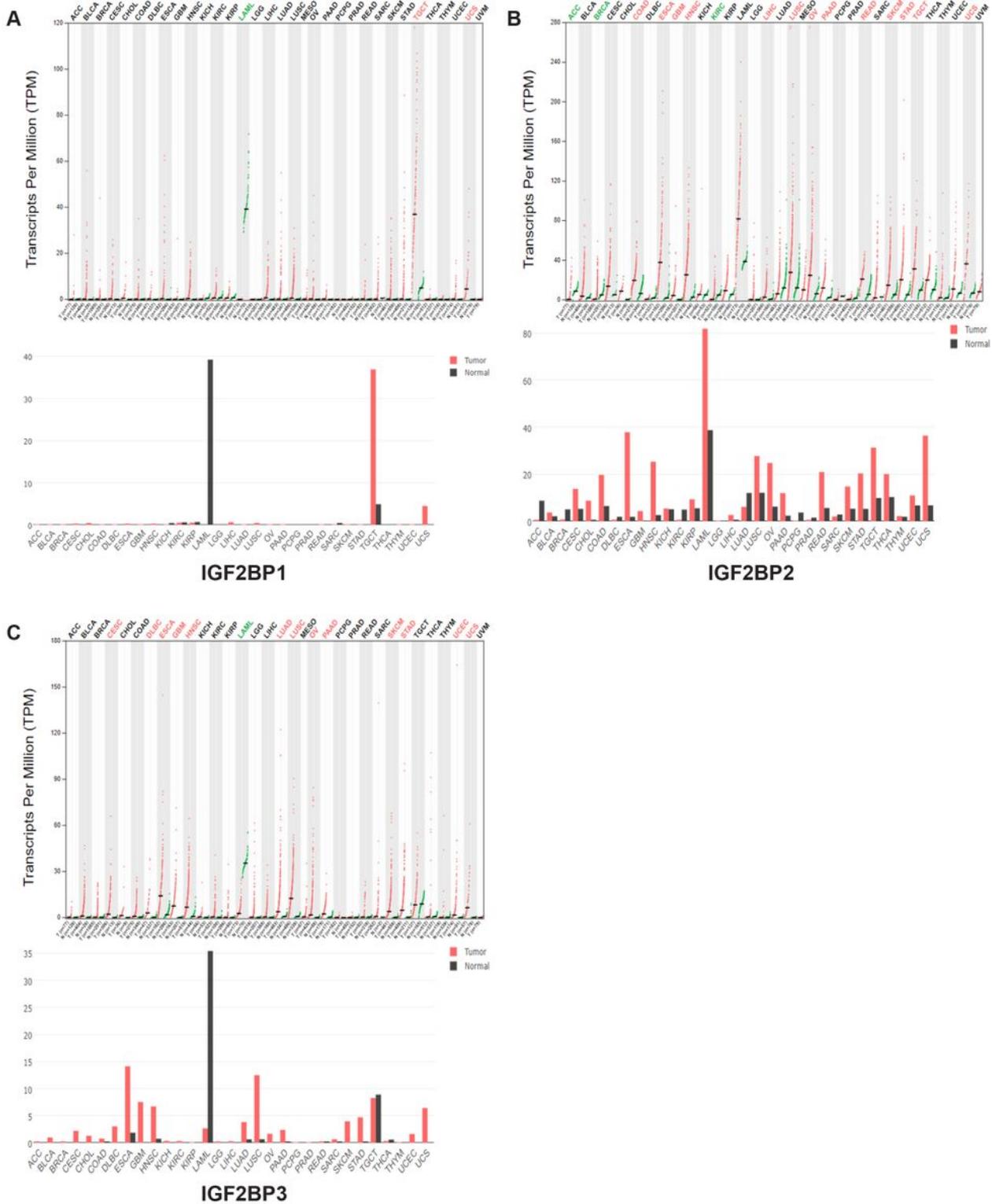
## Tables

Table 1

The Significant Changes of IGF2BP Expression in Transcription Level between Different Types of Pancreatic Cancer (ONCOMINE Database)

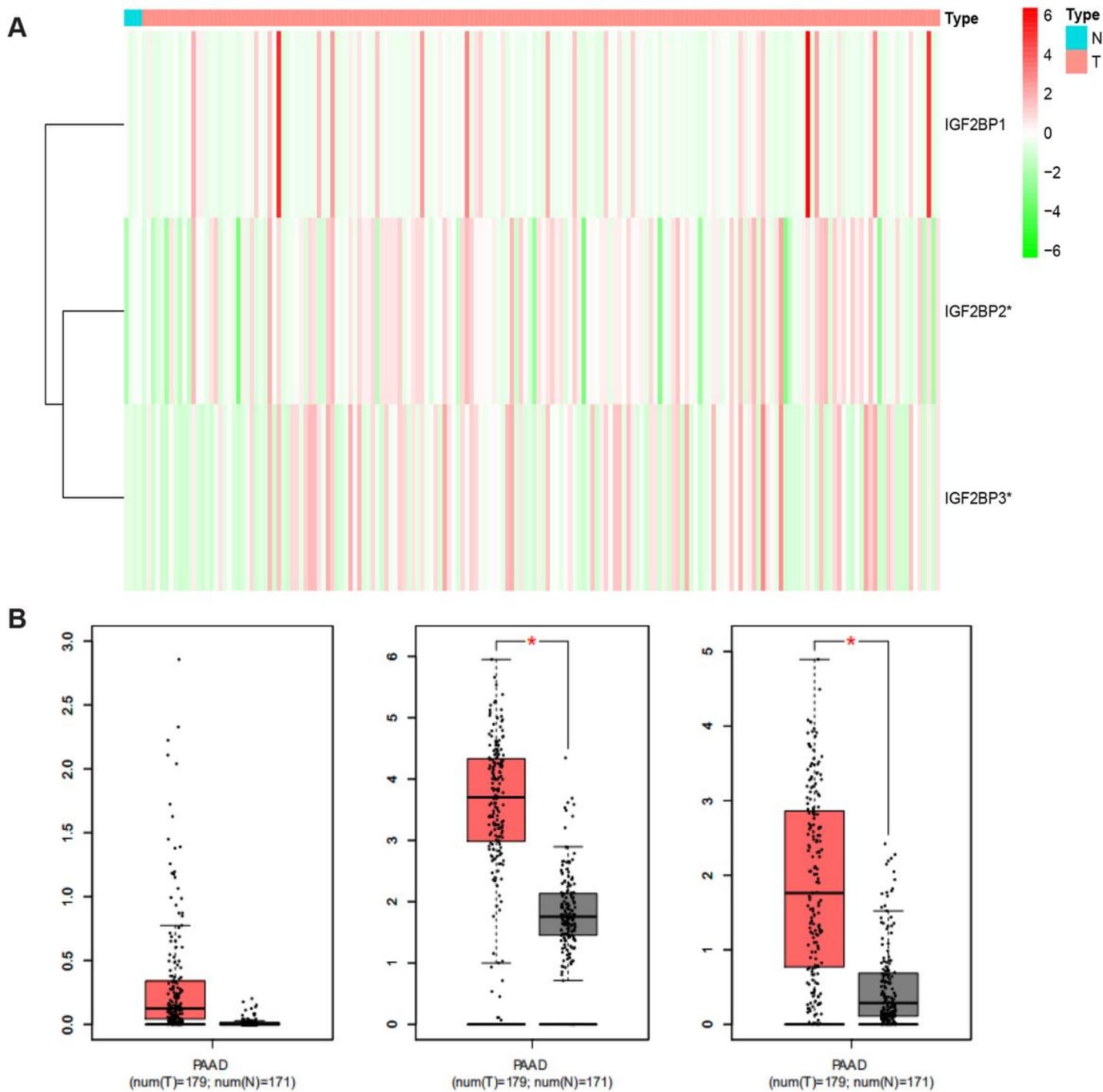
Gene ID	Types of Pancreatic Cancer versus Normal	Fold Change	p Value	t Test	References
IGF2BP2	Pancreatic Carcinoma versus Normal	3.446	1.90E-06	7.957	Segara Pancreas Statistics <sup>12</sup>
	Pancreatic Carcinoma versus Normal	2.657	1.93E-07	6.193	Pei Pancreas Statistics <sup>13</sup>
	Pancreatic Ductal Adenocarcinoma versus Normal	2.01	5.63E-09	6.407	Badea Pancreas Statistics <sup>14</sup>
IGF2BP3	Pancreatic Carcinoma versus Normal	9.327	4.11E-12	9.295	Pei Pancreas Statistics <sup>13</sup>
	Pancreatic Ductal Adenocarcinoma versus Normal	3.528	1.61E-08	6.643	Badea Pancreas Statistics <sup>14</sup>
	Pancreatic Ductal Adenocarcinoma versus Normal	2.355	0.005	2.725	Ishikawa Pancreas Statistics <sup>15</sup>
	Pancreatic Ductal Adenocarcinoma Epithelia versus Normal	5.606	0.007	2.703	Grutzmann Pancreas Statistics <sup>16</sup>

## Figures



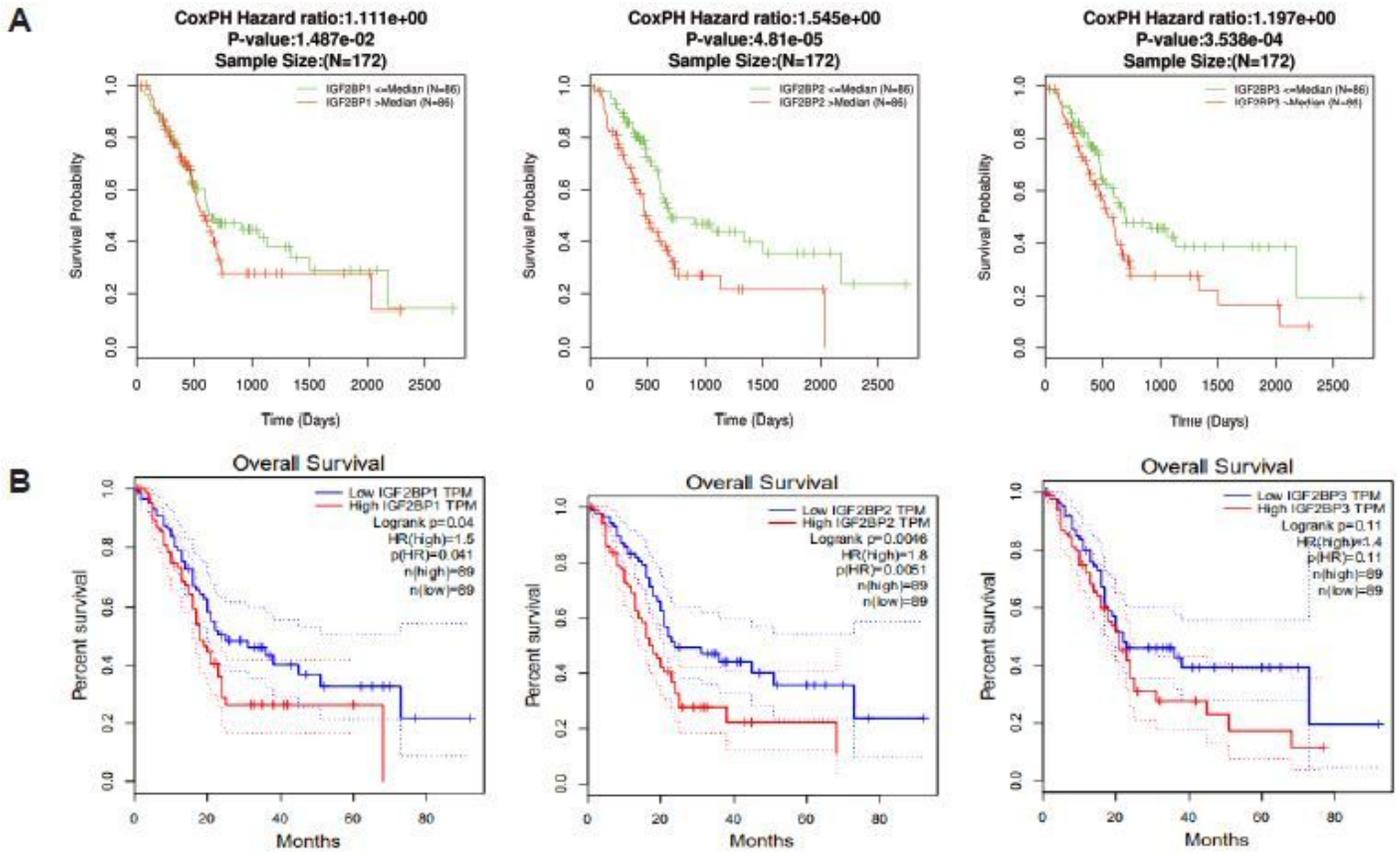
**Figure 1**

The Transcription Levels of IGF2BP Factors in Different Types of Cancers (GEPIA). (A-C) The expression of IGF2BPs in pan-cancer.



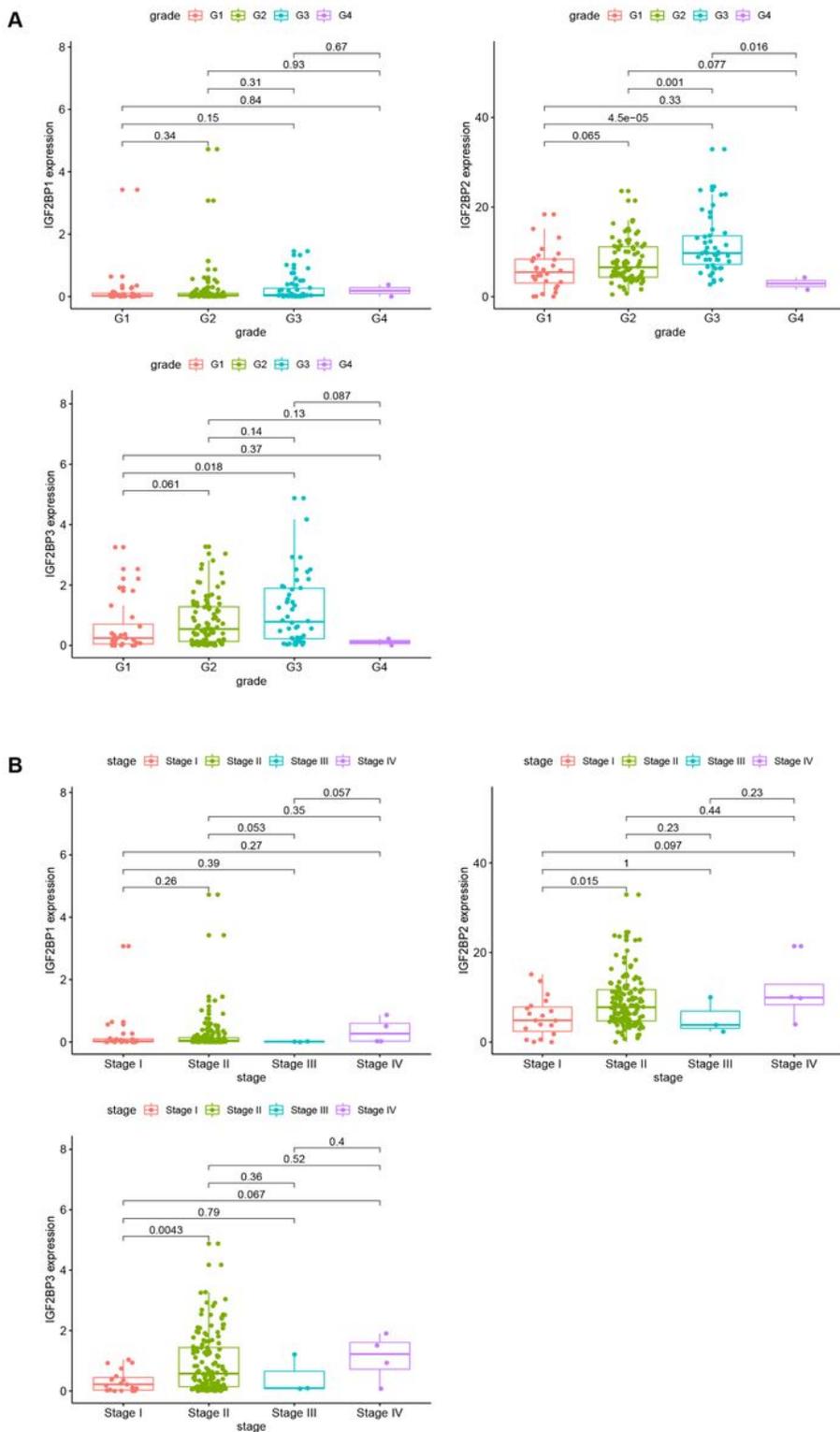
**Figure 2**

The Expression of IGF2BPs in Pancreatic Cancer (TCGA and GEPIA). (A) The expression of IGF2BPs in Pancreatic Cancer, analyzed by TCGA. (B) The expression of IGF2BPs in Pancreatic Cancer, analyzed by GEPIA.



**Figure 3**

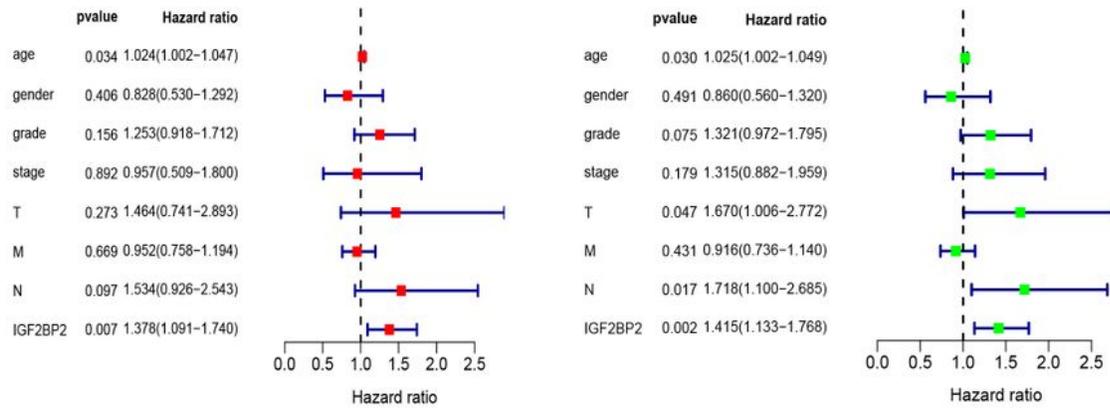
The Prognostic Value of mRNA Level of IGF2BP Factors in Pancreatic Cancer Patients (LinkedOmics and GEPIA). (A) The prognostic value of mRNA level of IGF2BP Factors in Pancreatic Cancer Patients, analyzed by LinkedOmics. (B) The prognostic value of mRNA level of IGF2BP Factors in Pancreatic Cancer Patients, analyzed by GEPIA.



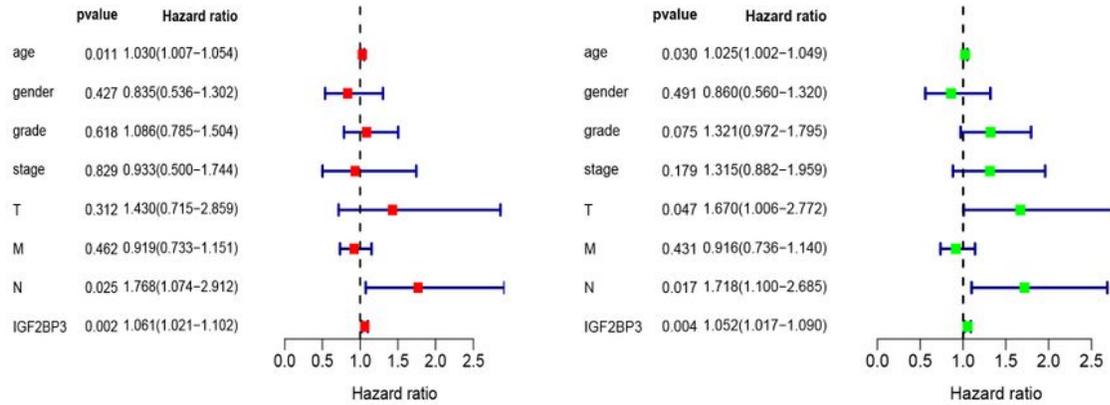
**Figure 4**

Correlation analysis between Grade&Stage and IGF2BP1-3 expression in 178 pancreatic cancer cases. (A) Correlation analysis between pathological grade and IGF2BP1-3 expression in 178 pancreatic cancer cases. (B) Correlation analysis between clinical stage and IGF2BP1-3 expression in 178 pancreatic cancer cases.

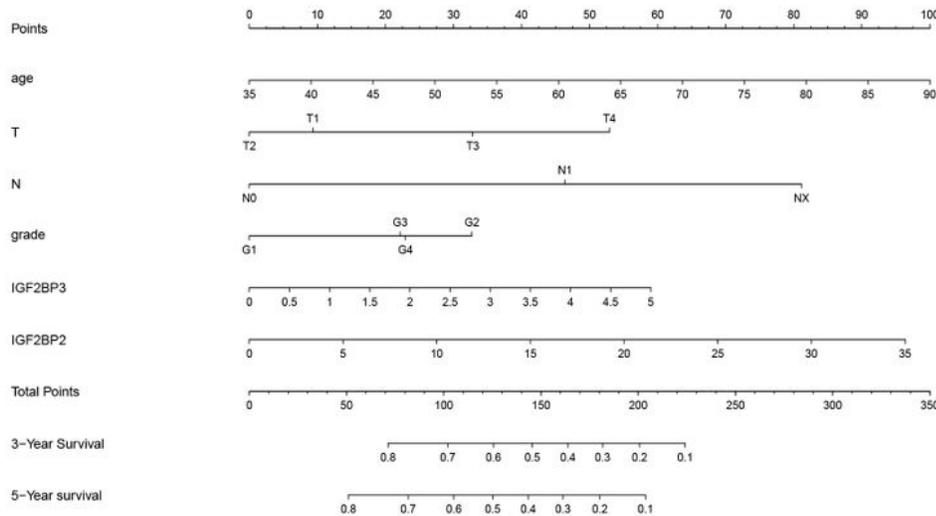
**A**



**B**

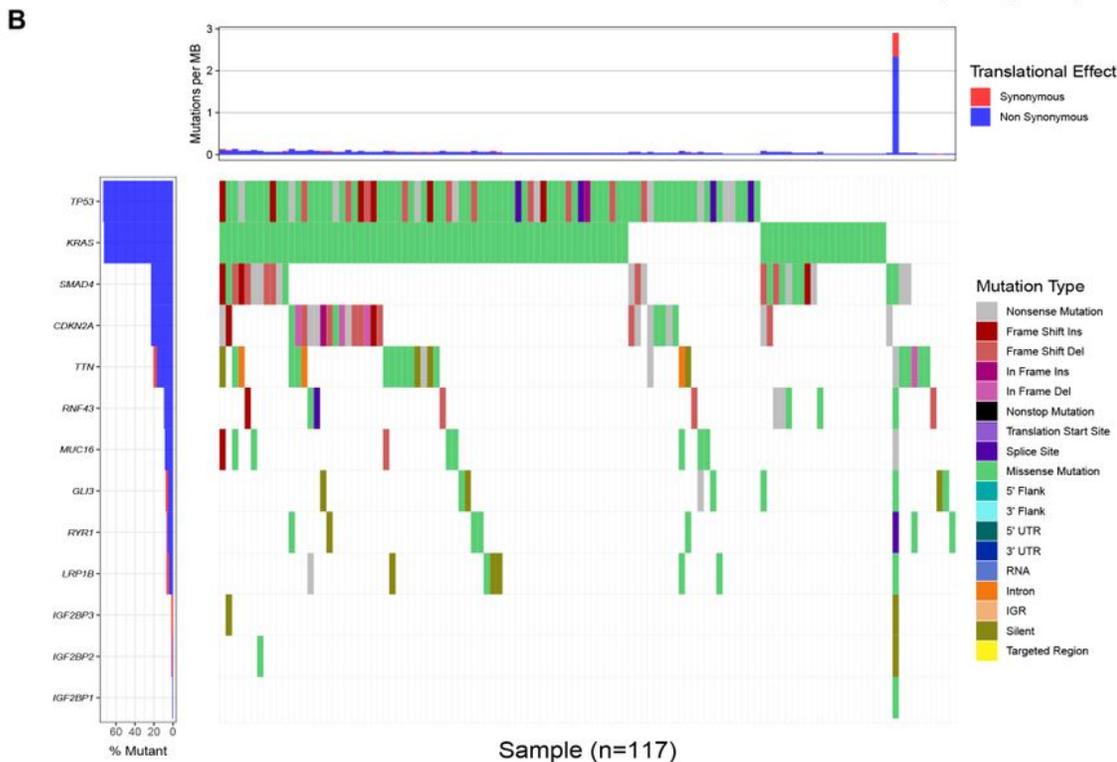
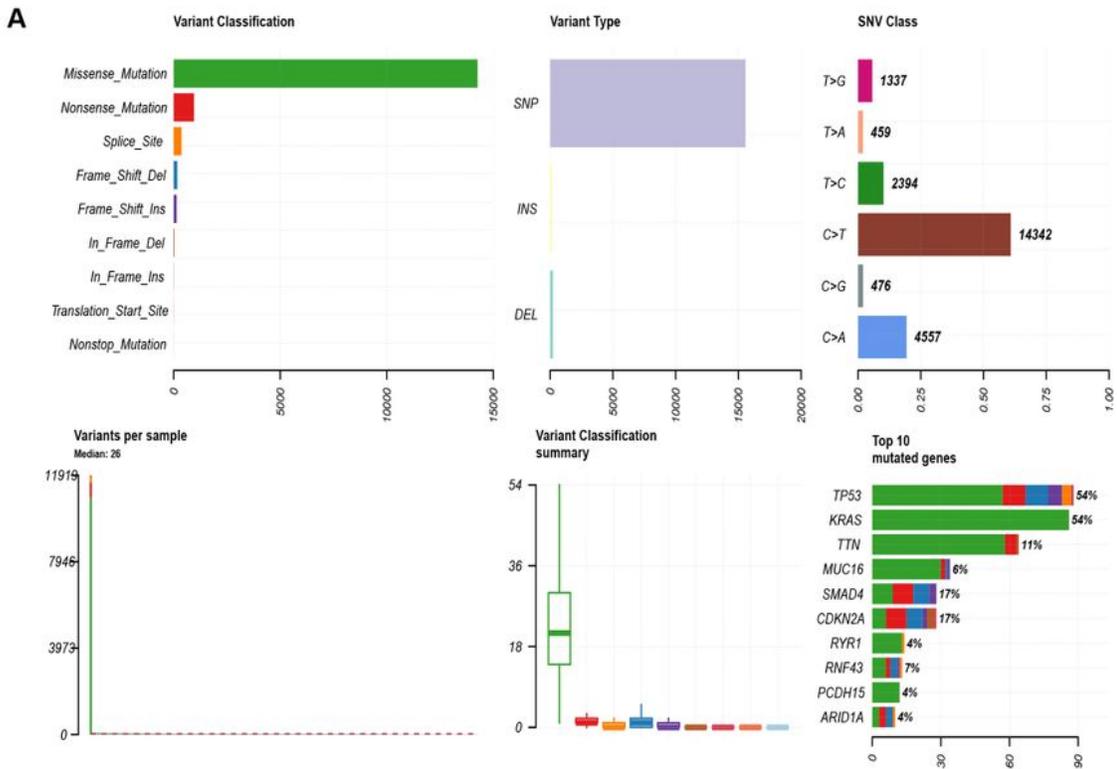


**C**



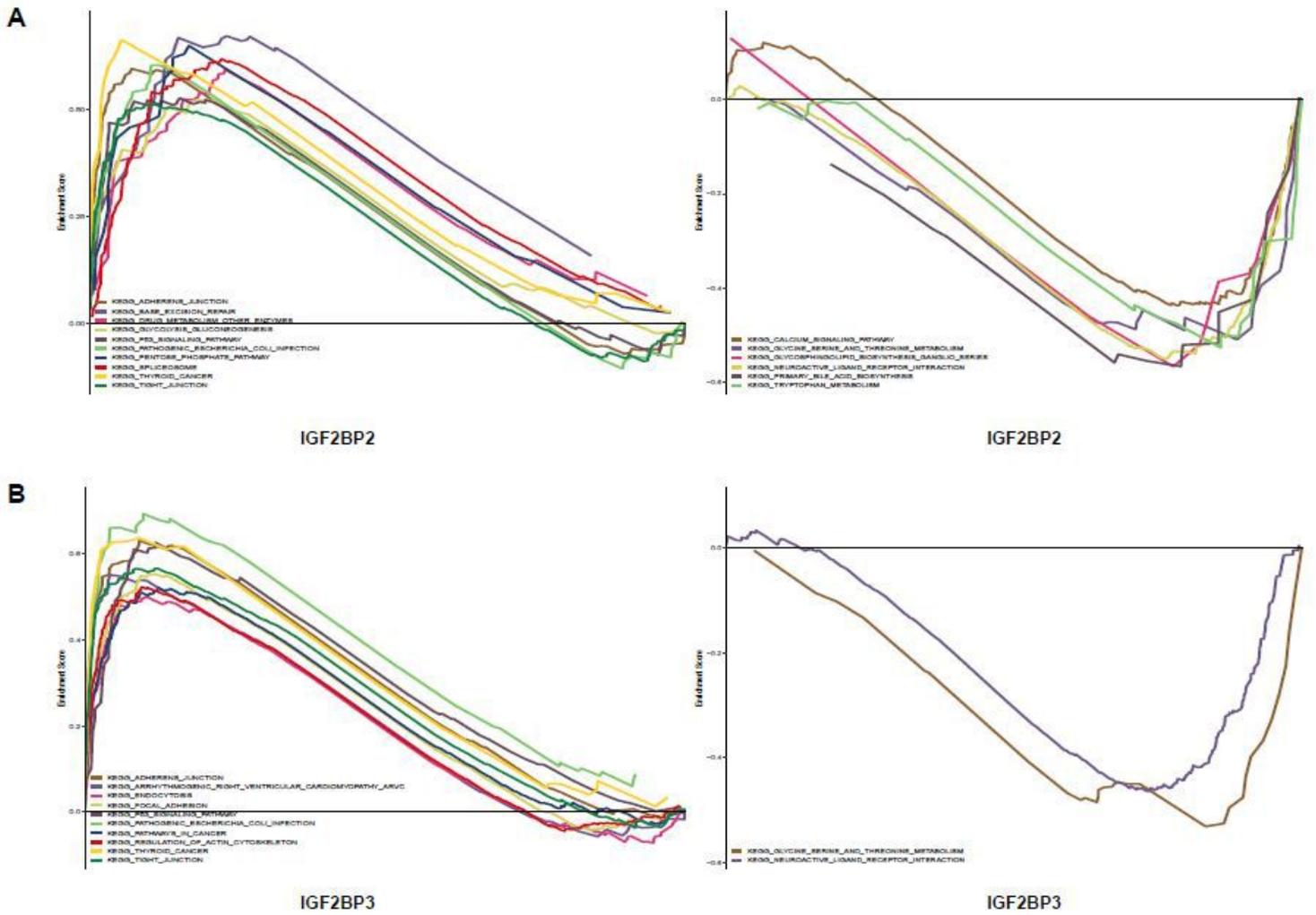
**Figure 5**

Cox's proportional hazard model of correlative factors in Pancreatic Cancer Patients. (A) Regarding IGF2BP2, univariate and multivariate COX regression analysis for factors affecting the overall survival. (B) Regarding IGF2BP3, univariate and multivariate COX regression analysis for factors affecting the overall survival (C) An established nomogram to predict survival based on COX model.



**Figure 6**

Gene mutation information in pancreatic cancer. (A) Missense mutation was detected to be the most frequent mutation classification in pancreatic cancer. SNP and C>T were confirmed to be the most fundamental Variant Type and SNV class. The median variation for each sample is approximately 26. The top 10 mutations in pancreatic cancer. (B) The mutation types of IGF2BP1-3 compared with the top 10 mutation types in pancreatic cancer.



**Figure 7**

GSEA enrichment analysis of the IGF2BP2 and IGF2BP3. (A) The up-regulated and down-regulated enriched pathway of IGF2BP2. (B) The up-regulated and down-regulated enriched pathway of IGF2BP3.

A

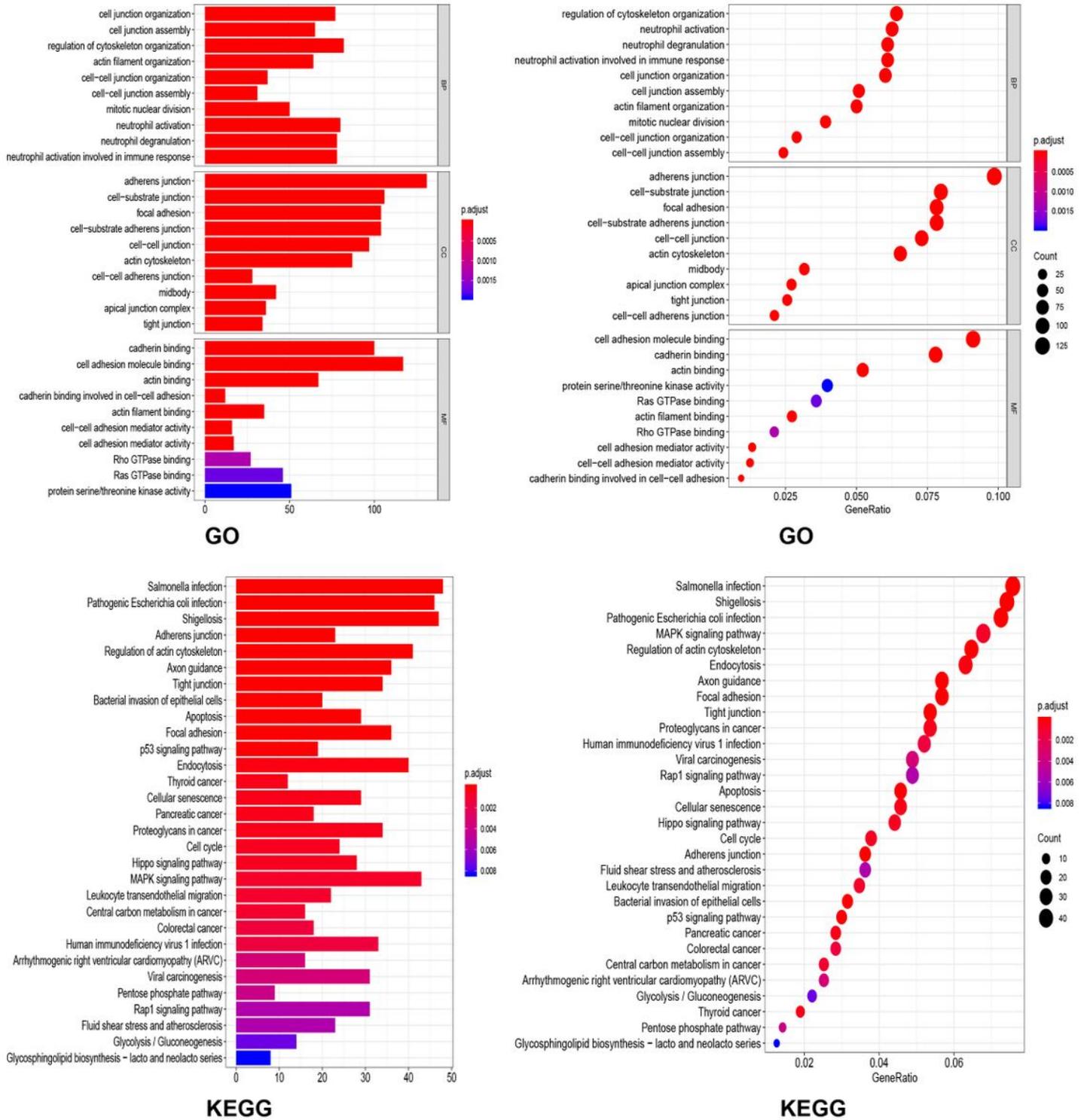
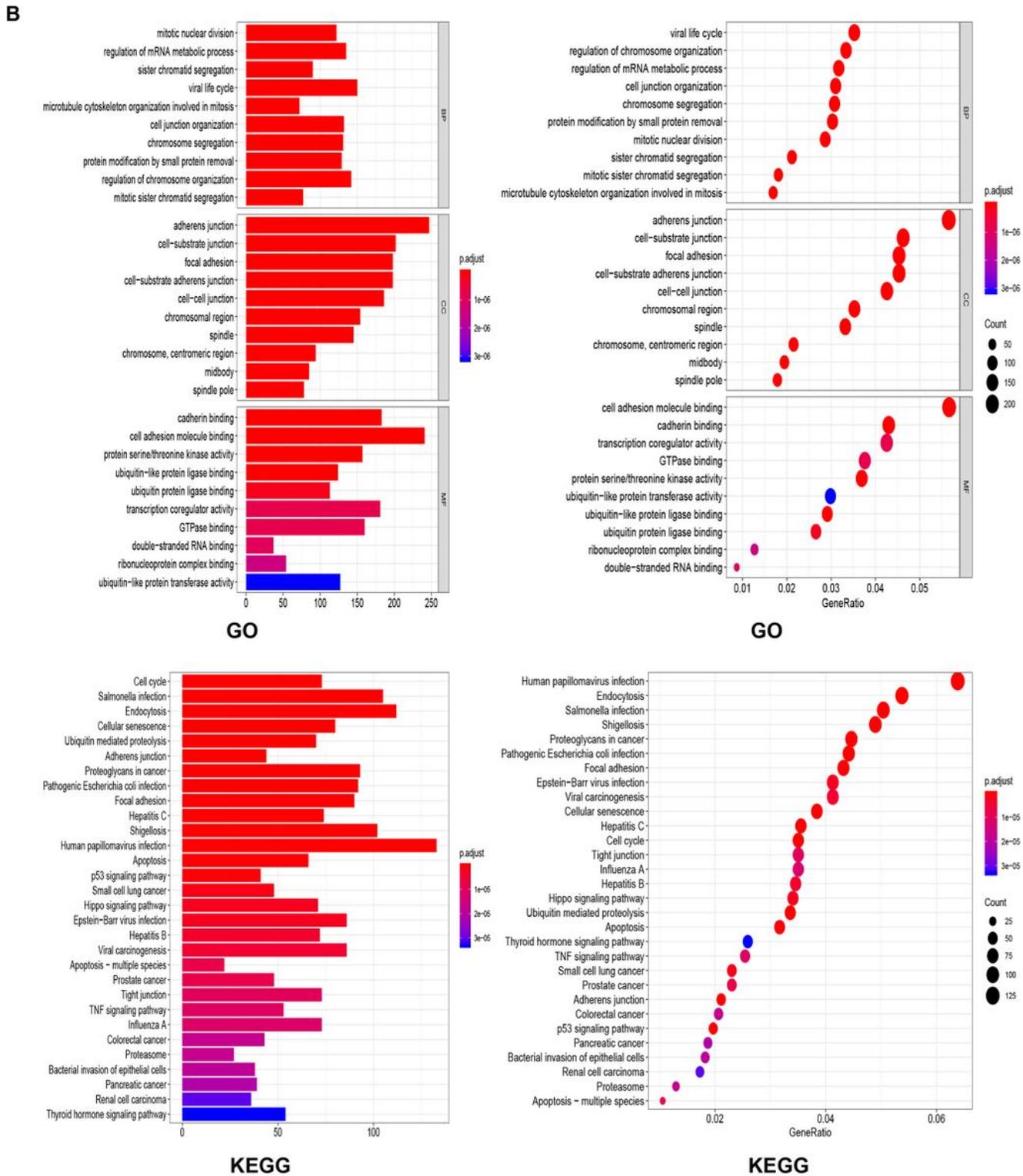


Figure 8

GO and KEGG enrichment analysis of the IGF2BP2 and IGF2BP3. IGF2BP2 and IGF2BP3, differentially expressed genes; GO, gene ontology; KEGG, Kyoto Encyclopedia of Gene and Genome.



**Figure 9**

A. Expression of IGF2BP2 and IGF2BP3 in pancreatic cancer and normal cell lines. B. Cell proliferation of SW1990 and ASPC detected by CCK8. C. Cell proliferation of SW1990 (NC vs siRNA) detected by plate clone formation assay. D. Cell invasion of SW1990 (NC vs siRNA) detected by Transwell assay (Magnification 400x).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SuppFig1.jpg](#)
- [supplementfigure.pdf](#)