

High Expression of AMIGO2 Is an Independent Predictor of Poor Prognosis in Pancreatic Cancer

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Research

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Abstract

Background.The AMIGO2 extracellular domain has a leucine - rich repetitive domain (LRR) and encodes a type 1 transmembrane protein , and is a member of the AMIGO gene family .Although the abnormal expression of AMIGO2 is associated with multiple tumors , the relationship with pancreatic cancer is not clear .

Methods.The expression of AMIGO2 mRNA and proteins in pancreatic cancer was analyzed using NCBI GEO database and GEPIA2 Human Protein Atlas database.The RNA sequencing data of pancreatic cancer and clinical data of pancreatic cancer patients in TCGA public database were retrospectively analyzed. AMIGO2 gene expression data and their corresponding clinical information in the sample were analyzed retrospectively. The diagnostic value of AMIGO2 expression in pancreatic cancer patients was determined by receiver operating characteristic (ROC) curve analysis. The effects of AMIGO2 expression differences on survival time of pancreatic cancer patients were analyzed by Kaplan-Meier Plotter database and GEPIA2 database.The correlation between AMIGO2 gene and TP53 mutation in pancreatic cancer was analyzed by UALCAN database and TIMER database. The similar genes of AMIGO2 in pancreatic cancer were analyzed by GEPIA2 database, and the biological behavior, cellular composition, molecular function enrichment analysis and protein interaction of similar genes were analyzed by DAVID database and Metascape database. enrichment analysis of AMIGO2 similar gene pathways using KEGG database. The MSIGDB cancer coexpression module in Harmonizome database and TIMER database were used to study the gene coexpression of AMIGO2 in pancreatic cancer. AMIGO2 transcription factors were predicted using the JASPER database. The pathway of AMIGO2 transcription factors and co-expression genes was studied by KEGG database.

Results. The expression of AMIGO2 (GSE16515, GSE15471) in pancreatic cancer tissues was significantly higher than that in normal tissues ($P < 0.05$). The GEPIA2 database also confirmed that the expression of AMIGO2 in pancreatic cancer tissues was significantly higher than that in normal tissues. The expression level of AMIGO2 gene was correlated with lymph node metastasis and histological grade of pancreatic cancer ($P < 0.05$). The high expression of AMIGO2 protein in pancreatic cancer was confirmed in Human Protein Atlas database. The overall survival rate and progression-free survival rate of pancreatic cancer patients with high expression of AMIGO2 were significantly shorter than those of patients with low expression of AMIGO2 in Kaplan-Meier Plotter database and GEPIA2 database. In the gene ontology analysis, it is found that AMIGO2 is involved in cell adhesion, proliferation, migration, apoptosis and other biological processes. KEGG analysis pathway is concentrated in the focal adhesion pathway, mitotic cell cycle changes, and the regulation of cell protein localization. Abnormal expression of AMIGO2 was found in pancreatic cancer caused by TP53 mutation in UALCAN and TIMER databases.In JASPAR database, we predicted that there are 10 transcription sites between AMIGO2 and transcription factor MYB. In addition, there are positive genes related to AMIGO2 in TIMER database and transcription factor MYB regulates tumor cell proliferation and apoptosis in PI3K-Akt signal transduction pathway and WNT signal pathway in pancreatic cancer.

Introduction

Pancreatic cancer is one of the highly malignant tumors of the digestive system. Although the prognosis has been slightly improved in recent years, the 5-year survival rate is still less than 8% [1]. Although pancreatic cancer is not among the top five related deaths in China, its proportion in cancer-related deaths has increased by 9% in the past 10 years, and this proportion has increased sharply with the change of lifestyle and dietary habits of Chinese residents as well as the acceleration of population aging [2]. At present, the pathogenesis of pancreatic cancer is still unclear, which brings great limitations to the treatment of pancreatic cancer. In addition, serum tumor markers such as CA199 have poor specificity and sensitivity in the diagnosis of pancreatic cancer [3]. Therefore, we need to study new markers of pancreatic cancer, hoping to improve the specificity and sensitivity of pancreatic cancer diagnosis. AMIGO is originally a novel sequence induced by the neuron-promoting protein amphoterin [4-5]. The AMIGO2 gene is located on human chromosome 12q13 and is a member of the Amigo gene family that encodes type 1 transmembrane proteins. The extracellular region of AMIGO2 has a leucine-rich repetitive (LRR) domain [6]. Studies have shown that genes with LRR domain are involved in regulating the growth, invasion and metastasis of tumor cells. The expression of LRR3b, a member of the LRR super family, is significantly down-regulated in human breast cancer [7], glioma [8], prostate cancer [9], and lung cancer [10]. Another member of the LRR super family, LGR5 has also been reported to be expressed in liver cancer [11], gastric cancer [12], basal cell carcinoma [13], colon cancer and ovarian cancer [14]. Overexpression of LGR5 promotes tumor cell proliferation [13], while silencing its expression induces tumor cell apoptosis [12]. In addition, studies have shown that the LRR domain of AMIGO2 plays a role in the process of collagen adhesion and migration of gastric cancer cells [15]. However, the role of AMIGO2 in pancreatic cancer has been rarely reported at home and abroad. For this purpose, we used the GEPIA2 [16] database, UALCAN [17] database, Kaplan-Meier plotter database [18], Human protein Atlas database [19], TIMER database [20], David database [21], and Metascape database [22], Harmonizome database [23], and Jaspas database [24] were used to comprehensively analyze the expression and prognosis of AMIGO2 and its proteins in pancreatic cancer. The possible mechanisms of AMIGO2 gene in pancreatic cancer were further studied. This study provides a theoretical basis for further study of AMIGO2 gene in pancreatic cancer.

Materials & Methods

1.1 Validation using the Gene Expression Omnibus (GEO), GEPIA2 and Human Protein Atlas (HPA) databases

GEO database (<http://www.ncbi.nlm.nih.gov/geo/>) GEO is an international public repository, which can archive and distribute microarrays, next-generation sequencing and other forms of high-throughput functional genomics data submitted by research groups free of charge. The gene expression profile data set GSE16515 [25] GSE15471 [26] was downloaded from it. GSE16515 and GSE15471 adopt the platform ([HG-U133_Plus_2] Affymetrix human genome U133 Plus 2.0 array). GSE16515 contains gene expression microarray data from 36 pancreatic cancers and 16 paracancerous tissues. GSE15471 contains gene

expression microarrays from 39 pancreatic cancers and 39 paracancerous tissues. The R language was used to preprocess the original data, and the probe was transformed according to the annotation information of the chip platform, so as to obtain the expression matrix data of GSE16515 and GSE15471, from which the expression value of AMIGO2 was extracted. The ROC model was used to analyze its diagnostic efficacy for pancreatic cancer.

GEPIA2 (<http://gepia.cancer-pku.cn/>) is a data visualization website for analyzing RNA-Seq expression data from TCGA and GTEx projects. The gene expression of AMIGO2 in pancreatic cancer (PAAD) and non-tumor tissues was compared by GEPIA2 study. The data screening conditions are: (1) tumor type: pancreatic cancer; (2) tissue comparison: pancreatic cancer tissue vs normal pancreatic tissue; (3) data type mRNA; (4) significance: $P < 0.01$; (5) Differential expression level: more than 2 times;

Human Protein Atlas (<https://www.proteinatlas.org>) is a website that contains the data based on the expression of immunohistochemical, including nearly 20 kinds of common malignant tumors. Users can identify specific protein expression patterns that are differentially expressed in specific types of tumors. In this study, immunohistochemical images were used to directly compare the protein expression of AMIGO2 in human normal pancreatic tissues and pancreatic cancer tissues.

1.2 TCGA data analysis

The Human Cancer Genome Atlas (TCGA) was launched in 2006 by the National Cancer and Cancer Institute (NCI) and the National Human Genome Institute (NHGRI). Currently, there are more than 11,000 patients with clinical and genetic information for more than 20 tissue types and 30 cancers. We downloaded RNA sequencing data and clinical information of 182 pancreatic cancer patients from the TCGA public database (<http://cancergenome.nih.gov/>). The clinical data of 178 cases were complete. We collated RNA data from 178 cases of pancreatic cancer. Clinical information of pancreatic cancer patients was divided into high expression group ($n = 89$) and low expression group ($n = 89$) according to the median value of AMIGO2 expression in the matrix. The correlation between the expression of AMIGO2 and clinical information in the two groups was analyzed.

1.3 Study on the relationship between AMIGO2 and survival of pancreatic cancer patients

Kaplan-Meier plotter database data (www.kmplot.com) sources include GEO, EGA and TCGA. The main purpose of this tool is to find and validate biomarkers and survival and prognosis in cancer patients based on meta-analyses. We used mRNA sequencing data from pan-cancer analysis in this database to study the relationship between AMIGO2 in pancreatic cancer and overall survival rate and disease-free survival rate of pancreatic cancer patients. The GEPIA2 database can be used as an online analytical tool for differential gene analysis in tumors and normal tissues, enabling survival analysis of differential genes. We analyzed the association between AMIGO2 and survival in pancreatic cancer patients using this database.

1.4 Correlation between AMIGO2 gene and TP53 gene mutation.

UALCAN (<http://ualcan.path.uab.edu>) is based on the TCGA database of 31 cancer types of level 3 RNA-seq and clinical data of interactive network resources. It can be used to analyze the relative transcriptional expression of potential genes of interest between tumor and normal samples and the association of transcriptional expression with relevant clinicopathological parameters. In this study, mRNA expression of AMIGO2 in TP53 mutation and wild-type pancreatic cancer was studied by UALCAN database. TIMER (<https://cistrome.shinyapps.io/timer/>) is a reliable and intuitive tool that provides 185 systems to evaluate different immune cell infiltration and its clinical effect and genetic mutations related to the differences of gene expression. In our study, the "gene mutation module" was used to evaluate the differential expression of AMIGO2 in multiple tumors after TP53 mutation. Log₂-fold variation in the differential expression of each gene for each cancer type. High level mutations ($P < 0.05$, $\log_2 Fc > 0$, red) and low level mutations ($P < 0.05$, $\log_2 Fc < 0$, blue).

1.5 Study on the biological function of AMIGO2 related genes in pancreatic cancer.

Since structurally similar genes have similar functions in terms of biological functions, we analyzed and downloaded similar genes in AMIGO2 through GEPIA2 database. Bioprocess (BP) cell component (CC), molecular function (MF) and KEGG pathway were analyzed using David database for the genes with Pearson correlation coefficient > 0.4 . Metascape (<http://metascape.org>) is a reliable, intuitive tool for gene annotation and gene list enrichment analysis. Based on functional annotation of gene/protein lists, Metascape facilitates data-based decision analysis. In this study, "rapid analysis" was used to verify the biological functions of AMIGO2 and related genes and to analyze and study the protein interactions.

1.6 Prediction of transcription factors and pathway of AMIGO2 gene in pancreatic cancer.

Harmonizome is a collection of information about genes and proteins from 114 data sets from 66 online sources. The database extracts information from the original data set into attribute sheets that define significant associations between genes and attributes, where attributes can be genes, proteins, cell lines, tissues, experimental perturbations, diseases, phenotypes, or drugs, depending on the data set. Gene and protein identifiers map to the NCBI Entrez GENE symbols and attribute to the appropriate ontology. We also calculate gene-gene and attribute-attribute similarity networks from attribute lists. These attribute sheets and similarity networks can be integrated to perform multiple types of computational analysis for knowledge discovery and hypothesis generation. We queried the genes co-expressed by AMIGO2 in pancreatic cancer through the MSigDB cancer co-expression module of Harmonizome, and found 356 co-expressed genes. Then we used the Gene_Corr module of TIMER database to screen the genes coexpressed with AMIGO2 in pancreatic cancer and the Pearson correlation coefficient was greater than 0.5. JASPAR is an open accessible database containing collated non-redundant transcription factor (TF) binding profiles stored as the location frequency matrix (PFM) and TF flexible model (TFFM) of TF of multiple species in six classification groups. AMIGO2 transcription factor prediction via the JASPAR database

1.7 Statistical processing SPSS 22.0 statistical software was used for analysis. Measurement data were expressed as mean \pm standard deviation ($\bar{x} \pm s$). The comparison of mean was performed by t-test, and the comparison of counting data rates was performed by χ^2 test. Log-rank test was used for survival analysis, and $P < 0.05$ was considered statistically significant.

Results

2.1 Expression of AMIGO2 in pancreatic cancer Two datasets, GSE16515 (fig. 1 B) and GSE15471 (fig. 1 A), were studied in GEO database, which showed that AMIGO2 was highly expressed in pancreatic cancer. $*** P < 0.001$ ROC results showed that the area under the curve (AUC) was 0.972, and the 95% CI was 0.952-0.992, suggesting that AMIGO2 has a good diagnostic efficacy for pancreatic cancer (fig. 1 C). Fig. 2 shows the difference of AMIGO2 gene expression between pancreatic cancer and normal pancreatic tissue in GEPIA2 database ($* P < 0.05$). In fig. 3, we found that AMIGO2 protein was highly expressed in pancreatic cancer tissues, and no AMIGO2 protein staining was found in normal pancreatic tissues. Our results show that AMIGO2 transcription and protein expression are over-expressed in pancreatic cancer patients.

2.2 Correlation between AMIGO2 expression and clinicopathological parameters in patients with pancreatic cancer .

We examined the RNA sequencing data from 178 pancreatic cancer samples. The samples were divided into high expression group (n=89) and low expression group (n=89) according to the median expression value of AMIGO2. The correlation between AMIGO2 and the clinicopathological features of pancreatic cancer was analyzed according to the high and low expression level of AMIGO2. We found that the expression difference of AMIGO2 was correlated with lymph node metastasis and histological classification of pancreatic cancer ($P < 0.05$). There was no significant correlation with age, sex, tumor size, invasion, pathological stage and distant metastasis of pancreatic cancer patients. ($P > 0.05$).

2.3 Correlation between AMIGO2 expression difference and prognosis of pancreatic cancer patients

In Kaplan-Meier plotter database, we calculated the overall survival rate (OS) and progression-free survival rate (RFS) of pancreatic cancer patients with high expression and low expression of AMIGO2. Fig.4-A OS with high expression of AMIGO2 in pancreatic cancer patients is obviously lower than OS with low expression. Risk ratio (HR) =2.23, logrankP=0.00019. Fig. 4-B RFS with high AMIGO2 expression in pancreatic cancer patients were significantly lower than those with low amigo2 expression. Risk ratio (HR)=4.2, logrankP=0.00023. Fig. 4-C and fig. 4-D respectively show that OS with high expression of AMIGO2 in pancreatic cancer patients is significantly lower than OS with low expression in GEPIA2 database. Risk ratio (HR) =2.1, logrankP=0.00045. The RFS with high AMIGO2 expression in pancreatic cancer patients were significantly lower than those with low amigo2 expression. Risk ratio (HR)=1.6, logrankP=0.027. From the analysis of the two databases, we can see that pancreatic cancer patients with high expression of AMIGO2 have worse prognosis.

2.4 AMIGO2 was significantly correlated with TP53 mutation in pancreatic cancer

We explored the difference of mRNA and protein expression between pancreatic cancer and normal tissues, and the patients with pancreatic cancer with high expression of AMIGO2 had worse prognosis. We further explored the correlation between AMIGO2 and TP53 mutation. In fig. 5-A, TIMER website uses TCGA database to confirm that TP53 mutation in pancreatic cancer is over 58%. Fig. 5-B shows the differential expression of AMIGO2 in various tumors after TP53 mutation in TIMER database. Log₂-fold change of AMIGO2 differential expression in each cancer type. PAAD (n = 170) Log₂(FC)=0.26, P<0.05, with positive correlation. The violin diagram in fig.5-D shows that the expression of AMIGO2 in TP53 mutant and wild pancreatic cancer is P<0.001 in the TIMER database. Fig. 5-C, We used UALCAN to further verify the expression values AMIGO2 normal tissues and TP53 mutant and wild-type AMIGO2 in pancreatic cancer tissues (***) P<0.001). No difference in expression of AMIGO2 was found between normal tissues and unmutated pancreatic cancer tissues.

2.5 Studies on the biological functions of AMIGO2 related genes in pancreatic cancer

We used GEPIA2 to analyze similar genes of AMIGO2 and download data. Pearson correlation coefficient >0.4 gene was used for functional enrichment analysis. Figure 6 is the analysis of biological process (BP) cell component (CC), molecular function (MF) and KEGG pathway of similar genes using David's website. In our study, it was found that the biological process (BP) of AMIGO2 similar genes was mainly enriched in GO: 0098609 cells through molecular adhesion. GO: 0045892 DNA-dependent negative regulation of transcription. GO: 0033627 One cell attaches to another cell or to a potential substrate via an integrin (a heterodimer adhesion receptor formed by the non-covalent binding of specific α and β subunits). GO: 0034394 The process by which proteins are transported to or maintained at sites outside the cell wall and/or plasma membrane. GO: 0043065 Any process that activates or increases the frequency, rate, or degree of cell death by the process of apoptosis. GO: 0050678 The process of regulating the frequency, rate, or degree of epithelial cell proliferation. GO: 0030335 Any process that activates or increases the frequency, rate, or degree of cell migration. GO: 0008283 The proliferation of cells leads to the expansion of the cell population. We found that AMIGO2 is involved in cell adhesion, proliferation, migration, apoptosis and other biological processes. Cell Component Analysis (CC) GO: 0005925 Anchoring cells to the extracellular matrix and forming a cell-substrate junction at the actin filament termination point. GO: 0005912 Intercellular junctions composed of epithelial cadherin-catenin complexes. The epithelial cadherin or E-cadherin of each interacting cell extends through the plasma membrane into the extracellular space and binds to each other. Cell Functional Analysis (MF) GO: 0005515 Protein Binding GO: 0098641 Any calcium-adhesive protein binding occurring during cell-cell adhesion. KEGG pathway is concentrated and enriched to 9 pathways, hsa:04510 focal adhesion. Hsa:05412 arrhythmia right ventricular cardiomyopathy. hsa:04810 regulation of actin cytoskeleton. hsa:05414 dilated cardiomyopathy. hsa:05202 Transcriptional disorders in cancer. hsa:04512 ECM- receptor interaction. hsa:04145 phagocyte. Hsa05222 small cell lung cancer. hsa:04390 Hippo signal path.

2.6 We used Metascape to demonstrate the functional enrichment and protein interaction of similar genes in AMIGO2, and constructed a network map of functional enrichment items. The biological function of AMIGO2 was further verified. The AMIGO2-related genes were enriched and analyzed in Metascape database. Fig. 7-a shows the top 20 notes with the most significant P values in enrichment analysis. GO:0034330 Cell connective tissue.hsa:04510 KEGG pathway focal adhesion pathway.Go: 0044772 Mitotic cell cycle changes.GO: 0042060 Wound healing.GO:1903827 Regulation of cellular protein localization. These pathways and biological behavior are associated with the AMIGO2 gene. Figure 7-B is a network diagram of functional enrichment items. The node of each color is a functional enrichment item, connected by similarity > 0.3 Figure 7-C shows the protein interaction network diagram and the interaction diagram of similar genes in different MCODE components.

2.7 we queried AMIGO2 co-expressed genes in cancer by Harmonizome MSIGDB cancer co-expression module and found 356 co-expressed genes. Then we used the TIMER database Gene_Corr module to screen the co-expressed genes and found that there were 10 genes co-expressed with the AMIGO2 gene and the correlation was greater than 0.5 in pancreatic cancer. In the TIMER database, we found that CDK2, MAP4K4, MMP14, PTPRU, BCL2L1, CDH3, ITGA2, CCND1, CTNNB1 and PRKC1 were positively correlated with AMIGO2 in pancreatic cancer, and the correlation coefficient was > 0.5, $P < 0.01$. Fig. 8 shows a scatter plot of AMIGO2 related genes in pancreatic cancer.

2.8 predict the pathway of AMIGO2 gene in pancreatic cancer by JASPAR database and KEGG database.

We obtained nucleotide sequences of AMIGO2 gene promoters in the NCBI database and predicted the most likely binding transcription factors of AMIGO2 genes using the ANIMAL3.0 website, The promoter sequence is the chain of justice, P less than 0.05, The top 20 transcription factors were screened. Using JASPAR website to verify that MYB and AMIGO2 have 10 binding sites, We selected the highest three binding sites for display. In addition, ITGA2, BCL2L1, CCND1, CDK2, and MYB were found to be involved in the PI3K-Akt signaling pathway. Figure 9 shows the binding site information of AMIGO2 gene and MYB. Figure 10 illustrates the possible signaling pathways of AMIGO2 in pancreatic cancer.

Discussion

Pancreatic cancer is a common malignant tumor of the digestive tract. Due to its difficulty in early detection and easy metastasis and recurrence, it is the main reason for poor quality of life and short survival of pancreatic cancer patients. This requires us to conduct in-depth research on the occurrence and development of pancreatic cancer, especially on the genes and proteins that play a key role in the pathogenesis and treatment of pancreatic cancer. With the maturity of genomics, transcriptomics and high-throughput sequencing technology and the improvement of bioinformatics database, we can explore the occurrence and development of pancreatic cancer from the direction of basic research. AMIGO is a novel sequence induced by amphoteric proteins promoted by neurites. AMIGO1, AMIGO2 and AMIGO3 together constitute a new gene family, encoding type I transmembrane proteins and containing six

leucine-rich repeats (LRR) [27]. Studies have shown that genes with LRR domain can induce the proliferation, migration and metastasis of a variety of tumors. In addition, the AMIGOS

family showed two conserved serine-rich regions; One is near the transmembrane domain and the other is at the end of the COOH. The COOH-terminal serine-rich region of AMIGO2 has a common sequence of casein kinase II serine/threonine kinase [28]. The transmembrane form of TNF- α has a common SXXS sequence, which is the substrate for casein kinase I-dependent phosphorylation [28]. AMIGO2 has four possible casein kinase I phosphorylation sites in both of these conserved serine-rich regions [27]. This suggests that AMIGO2 may bind to TNF- α . TNF α involved in the signaling pathway has been shown to play an important role in pancreatic tumors [29-31]. In addition, AMIGO2 is involved in the process of collagen adhesion and migration of gastric cancer cells [15]. AMIGO2 also mediates the adhesion of tumor cells to hepatic endothelial cells and promotes the metastasis of hepatoma cells in the liver [32]. These evidences indicate that AMIGO2 has the structural characteristics of participating in the proliferation, invasion, migration and distant metastasis of various malignant tumors, and plays an important role in the metastasis of liver cancer and gastric cancer.

In this study, it was found that mRNA and protein levels of AMIGO2 were significantly up-regulated in pancreatic cancer. Meanwhile, ROC curve analysis showed that AMIGO2 had good diagnostic value in pancreatic cancer and was a potential marker for early diagnosis of pancreatic cancer. Through the GEPIA2 database and Kaplan-Meier plotter database, we verified that pancreatic cancer patients with high expression of AMIGO2 had shorter overall survival rate and progression-free survival rate, respectively. These results suggest that AMIGO2 can be used as a prognostic indicator of pancreatic cancer. In clinical feature correlation studies, high expression of AMIGO2 was found to be significantly associated with lymph node metastasis and poorer histomathological grade. We further explored the reasons for the abnormal expression of AMIGO2 in pancreatic cancer and unexpectedly found that there was a correlation between the abnormal expression of AMIGO2 in pancreatic cancer and TP53 gene mutation. TP53 is one of the most common mutated genes in all cancers, and is mutated in 70% of pancreatic cancer [33]. Our study found that the high expression of AMIGO2 in pancreatic cancer was significantly correlated with TP53 mutation. There were significant differences in the expression of AMIGO2 between patients with TP53 mutation and those without TP53 mutation in pancreatic cancer. In addition, there were also differences in the expression of AMIGO2 between normal controls and pancreatic cancer patients with TP53 mutation. In a study of breast cancer, it was shown that high expression of AMIGO2 was significantly correlated with relapsed-free survival in patients with TP53 wild-type, but not with TP53 mutated breast cancer [34]. As the mutation of TP53 in tumors cannot be effectively controlled, AMIGO2, as the downstream target gene of patients with TP53 mutated pancreatic cancer, may provide a new therapeutic target for TP53 mutated pancreatic cancer by intervening with it.

The malignant degree of tumor is often closely related to the proliferation, apoptosis, migration and invasion of tumor. AMIGO2 plays a role in regulating apoptosis [35-37], adhesion migration [38], invasion [34] and metastasis [39]; in breast cancer, liver cancer, gastric cancer and melanoma and other malignant

tumors. Some scholars have also called AMIGO2 an anti-apoptotic gene^[40]. From this, we can see that AMIGO2 plays different roles in malignant tumors. This prompted us to further explore the biological behavior and signaling pathway of AMIGO2 in pancreatic cancer. The enrichment analysis of the interaction between AMIGO2 and its similar genes and proteins showed that the functions of AMIGO2 and its similar genes were mainly concentrated in molecular adhesion, cell apoptosis, proliferation, and interaction with epithelial cadherin or E-cadherin. Pathways are enriched in transcriptional dysregulation, regulation of actin cytoskeleton, focal adhesion, and ECM-receptor interactions in cancer. In the protein interaction network diagram, we can see that AMIGO2 also plays a role in cell adhesion and migration. In the study on the conduction mechanism of AMIGO2 in pancreatic cancer, we predicted the transcription factors of AMIGO2, and found that there were 10 binding sites between MYB and AMIGO2, and the three sites with the highest score and the most significant P value were displayed. We know that MYB is a proto-oncogene and a transcription factor that regulates cell proliferation, differentiation, and apoptosis through signaling pathways. Studies have shown that MYB is expressed in pancreatic cancer and regulates the malignancy of pancreatic cancer^[41-43]. Then we studied 10 genes that are co-expressed in AMIGO2 in pancreatic cancer pathway. Cyclin-dependent kinase 2 (CDK2), apoptosis regulator Bcl-X(Bcl2L1), G1/S specific cyclin D1(CCND1), and PRKCI regulate tumor by regulating cell cycle and apoptosis. In addition, ITGA2, BCL2L1, CCND1, CDK2 and MYB are involved in PI3K-Akt signaling pathway. Therefore, we believe that AMIGO2 may bind to MYB through PI3K-Akt signaling pathway and then regulate CDK2, CCND1, BCL2L1 and PRKCI, thus affecting the biological behavior of tumor cell proliferation and apoptosis invasion. In addition, we found that pancreatic cancer phosphatase 2 (PTPRU) binds to catenin β -1 (CTNNB1), and tyrosine protein phosphatase can dephosphorylate CTNNB1 phosphates to regulate CTNNB1 functions in cell adhesion and signaling, and plays a role in cell proliferation and migration^[44-46]. In addition, CTNNB1 is a key molecule downstream of the Wnt signaling pathway^[46] and is involved in cell apoptosis. These results suggest that AMIGO2 may also regulate the proliferation and apoptosis of pancreas through the Wnt pathway. In conclusion, the screened pathway can provide a theoretical basis for further research on the biological functions and related mechanisms of AMIGO2 in pancreatic cancer.

Conclusions

In summary, we found that there was significant overexpression of AMIGO2 in pancreatic cancer, and that the overexpression of AMIGO2 was significantly correlated with TP53 mutation. The overexpression of AMIGO2 in pancreatic cancer patients predicts a shorter survival period and can be an independent prognostic factor. AMIGO2 may play a role in the biological processes of proliferation, apoptosis and invasion in pancreatic cancer through PI3K-Akt signaling pathway and Wnt signaling pathway. However, all the data analyzed in our study were retrieved from the online database, and further studies including larger samples and cell and animal experiments are needed to verify our findings, and the clinical application of AMIGO2 in the treatment of pancreatic cancer, especially pancreatic cancer caused by TP53 mutation, is also needed to be further explored.

Declarations

Ethics approval and consent to participate

As all the data were retrieved from the online databases, so it could be confirmed that all written informed consent had already been obtained.

Consent for publication

Not applicable

Availability of data and materials

The data of this study come from Publicly available datasets .These data can be found here: the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>) ,The Human Cancer Genome Atlas (<http://cancergenome.nih.gov/>),Kaplan-Meier plotter database data(www.kmplot.com),GEPIA2 (<http://gepia.cancer-pku.cn/>),Human Protein Atlas (<https://www.proteinatlas.org>),UALCAN (<http://ualcan.path.uab.edu>),TIMER (<https://cistrome.shinyapps.io/timer/>)and Metascape(<http://metascape.org>).

Competing interests

The authors declare that they have no competing interests

Funding

Not applicable

Authors' contributions

Zj complete the bioinformatics analysis, designed research and wrote draft .Fxf analyze the data and conduct a statistical analysis.All authors read and approved the final manuscript.

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Tables

Table 1. relationship between the AMIGO2 expression difference and clinicopathological features of pancreatic cancer

Characteristic	Low expression of AMIGO2	High expression of AMIGO2	p
n	89	89	
T stage, n (%)			0.097
T1	4 (2.3%)	3 (1.7%)	
T2	17 (9.7%)	7 (4%)	
T3	65 (36.9%)	77 (43.8%)	
T4	2 (1.1%)	1 (0.6%)	
N stage, n (%)			0.014
N0	33 (19.1%)	17 (9.8%)	
N1	54 (31.2%)	69 (39.9%)	
M stage, n (%)			1.000
M0	40 (47.6%)	39 (46.4%)	
M1	3 (3.6%)	2 (2.4%)	
Pathologic stage, n (%)			0.320
Stage I	14 (8%)	7 (4%)	
Stage II	69 (39.4%)	77 (44%)	
Stage III	2 (1.1%)	1 (0.6%)	
Stage IV	3 (1.7%)	2 (1.1%)	
Gender, n (%)			0.880
Female	39 (21.9%)	41 (23%)	
Male	50 (28.1%)	48 (27%)	
Age, n (%)			0.133
<=65	41 (23%)	52 (29.2%)	
>65	48 (27%)	37 (20.8%)	
Histologic grade, n (%)			0.033
G1	21 (11.9%)	10 (5.7%)	
G2	45 (25.6%)	50 (28.4%)	
G3	19 (10.8%)	29 (16.5%)	
G4	2 (1.1%)	0 (0%)	

Characteristic	Low expression of AMIGO2	High expression of AMIGO2	p
Age, mean \pm SD	66.27 \pm 9.26	63.22 \pm 12.01	0.060

Figures

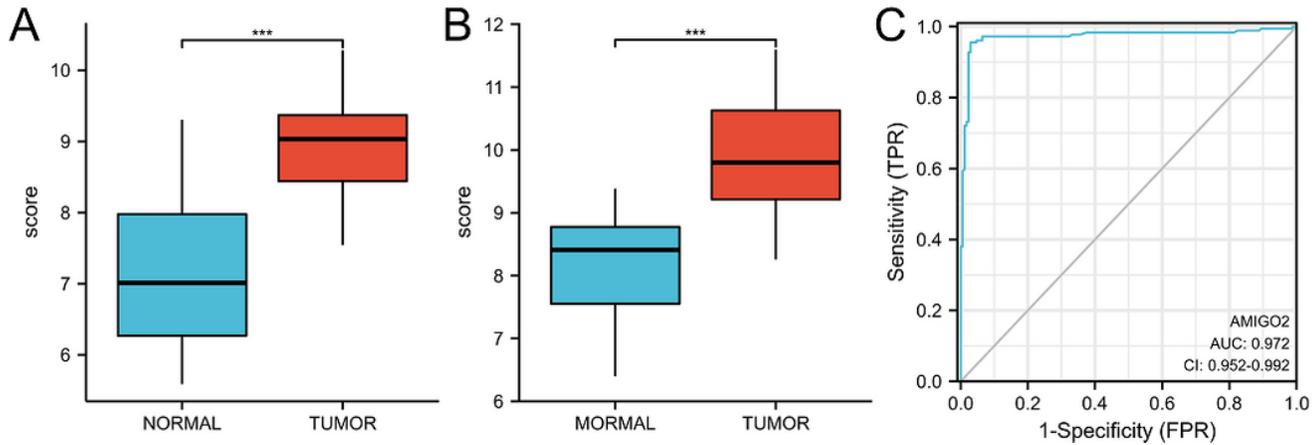
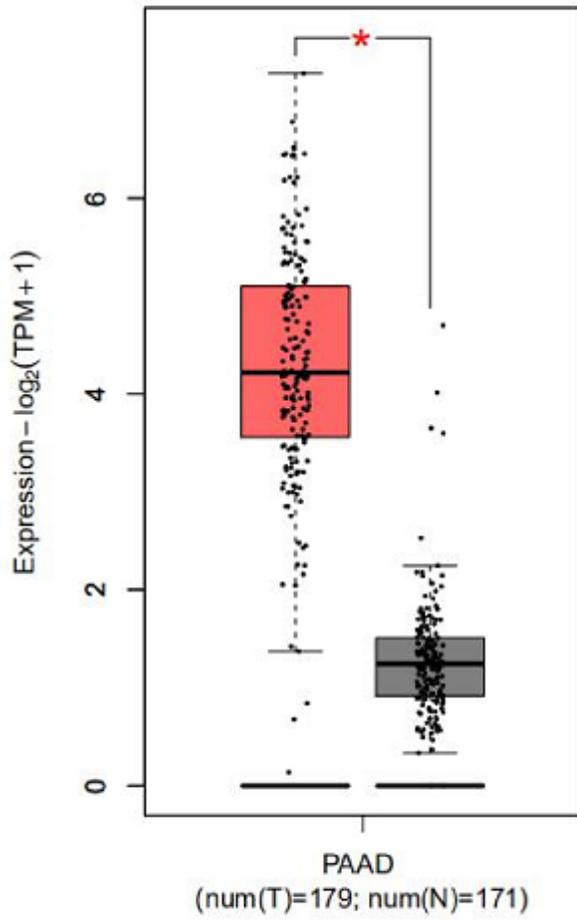
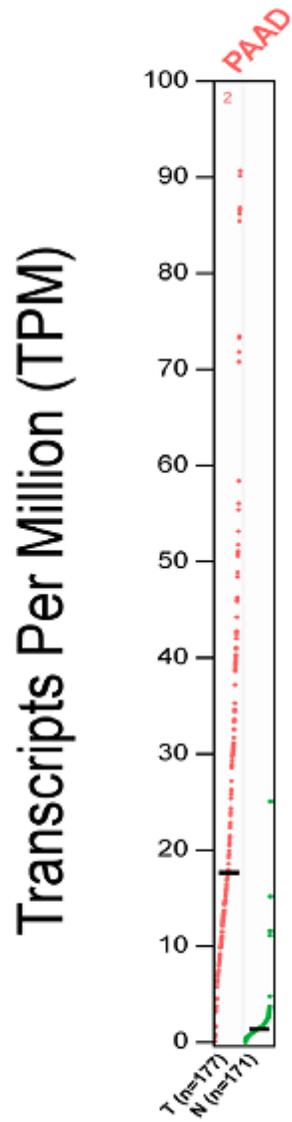


Figure 1

Differential expression of AMIGO2 in Pancreatic Cancer and adjacent Pancreatic Cancer in data set GSE16515, GSE15471 and Diagnostic efficacy Curve of AMIGO2. (A) AMIGO2 is highly expressed in the dataset GSE15471. (B) AMIGO2 is highly expressed in the dataset GSE16515. (C) Diagnostic efficacy curve of AMIGO2 in pancreatic cancer.

A**B****Figure 2**

The Expression of AMIGO2 in Pancreatic Cancer(GEPIA2)(A)box plot (B)scatter diagram

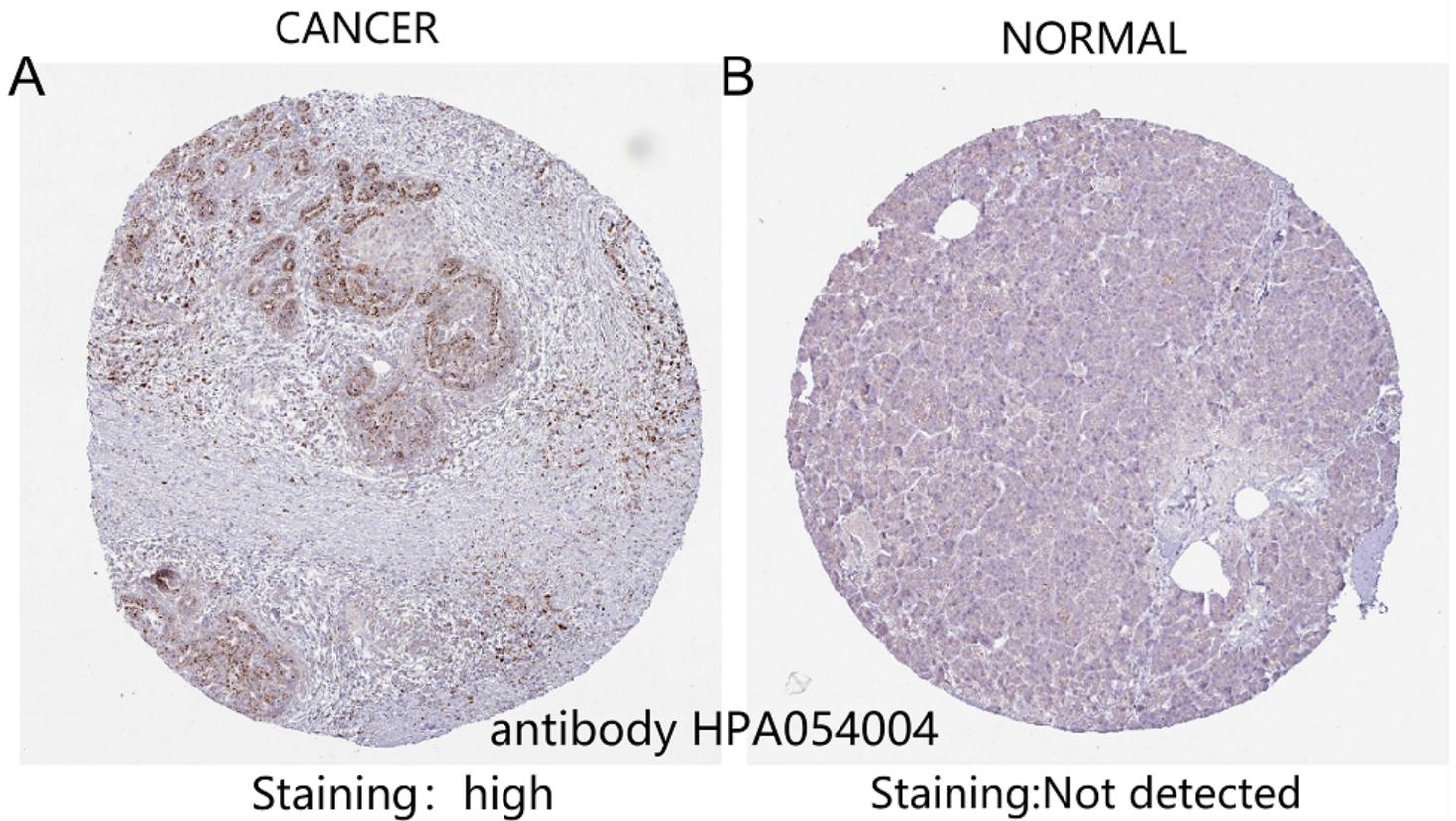


Figure 3

Representative immunohistochemical images of AMIGO2 in pancreatic and normal pancreatic tissues. (A) High expression of AMIGO2 protein in pancreatic cancer tissue. (B) AMIGO2 protein was not expressed in normal pancreatic tissue.

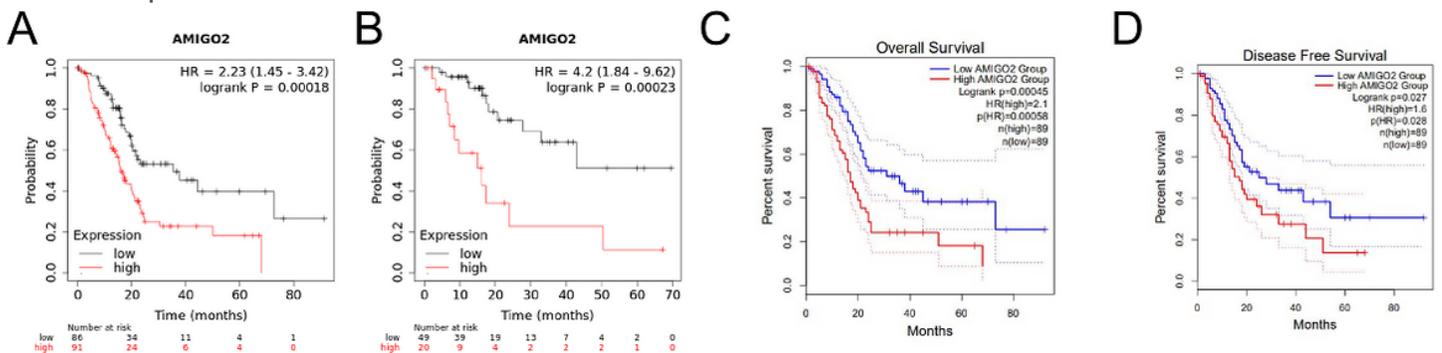


Figure 4

prognostic value of AMIGO2 mRNA expression in pancreatic cancer patients. Generally, AMIGO2 mRNA high expression is associated with poor OS and RFS in PAAD patients. OS and RFS in patients with AMIGO2-expression pancreatic cancer in Kaplan Meier Plotter database(A,B). OS and RFS in patients with AMIGO2-expression pancreatic cancer in GEPIA2 database(C,D).

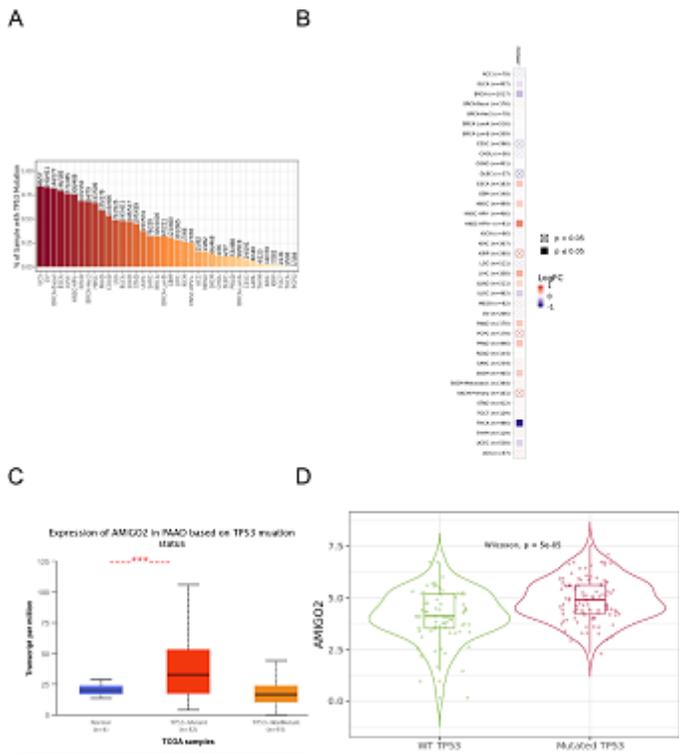


Figure 5

AMIGO2 high expression is significantly associated with TP53 mutations in pancreatic cancer. More than 58% of pancreatic cancer TP53 mutations in timer database(A). The differential expression of AMIGO2 in various tumors after TP53 mutation in TIMER database(B). UALCAN high expression in pancreatic cancer was significantly associated with TP53 mutations(C*** $p < 0.001$). High AMIGO2 expression in pancreatic cancer was significantly associated with TP53 mutations in the TIMER database(D).

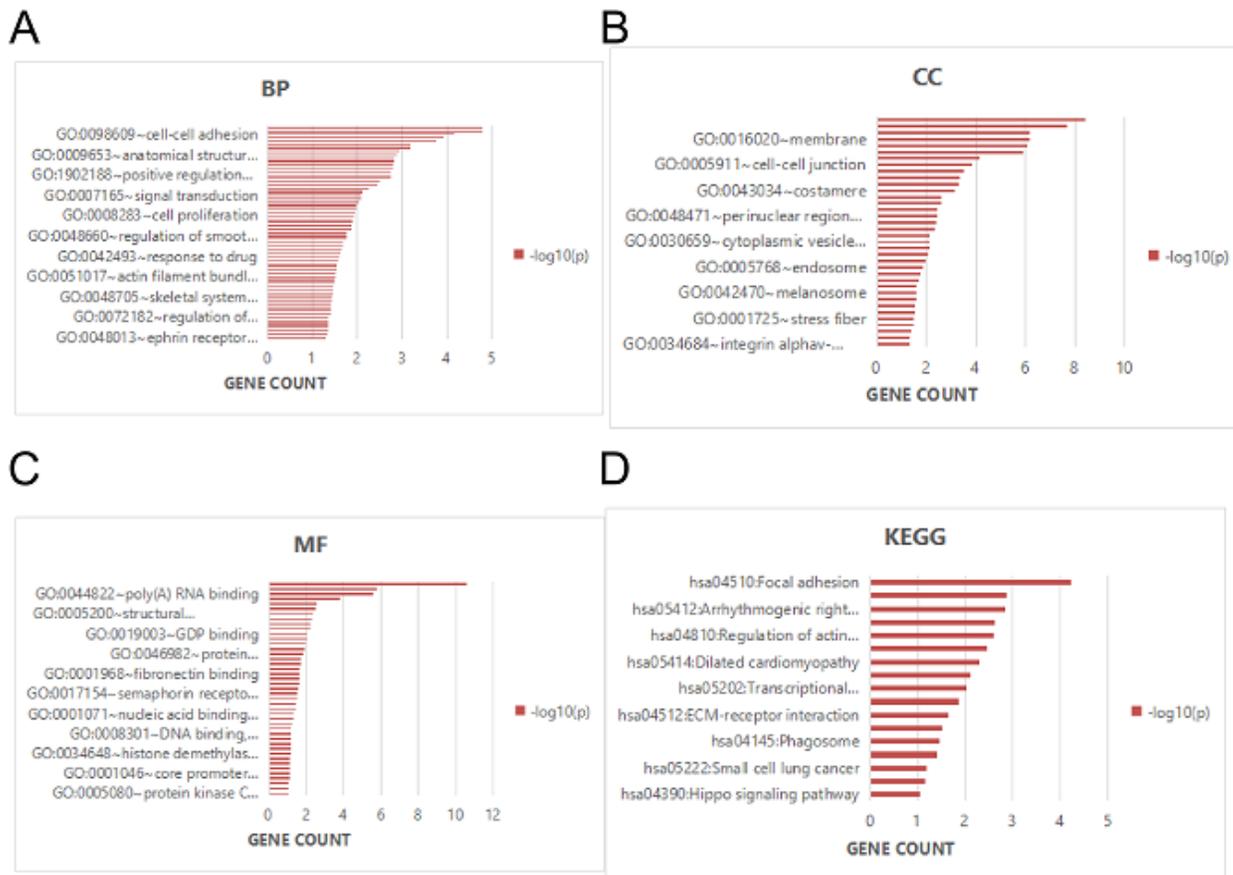


Figure 6

Predicted functions and pathways of AMIGO2 and its similar genes. the analysis of biological process (A) cell component (B), molecular function (C) and KEGG pathway (D) .

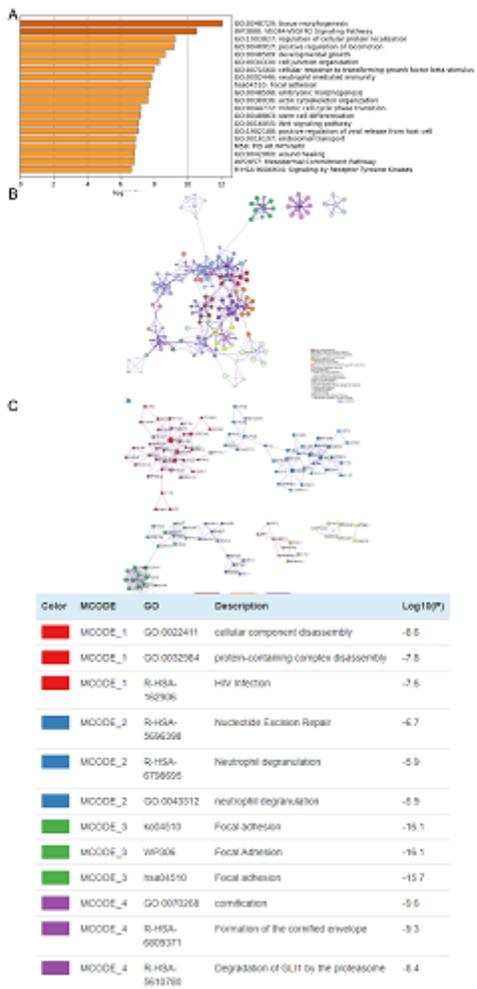


Figure 7

Prediction of the top 20 biological functions of AMIGO2 and its similar genes with the lowest p value by Metascape database. A network diagram of functional enrichment items. The node of each color is a functional enrichment item. B. protein interaction network diagram and the interaction diagram of similar genes in different MCODE components. C.

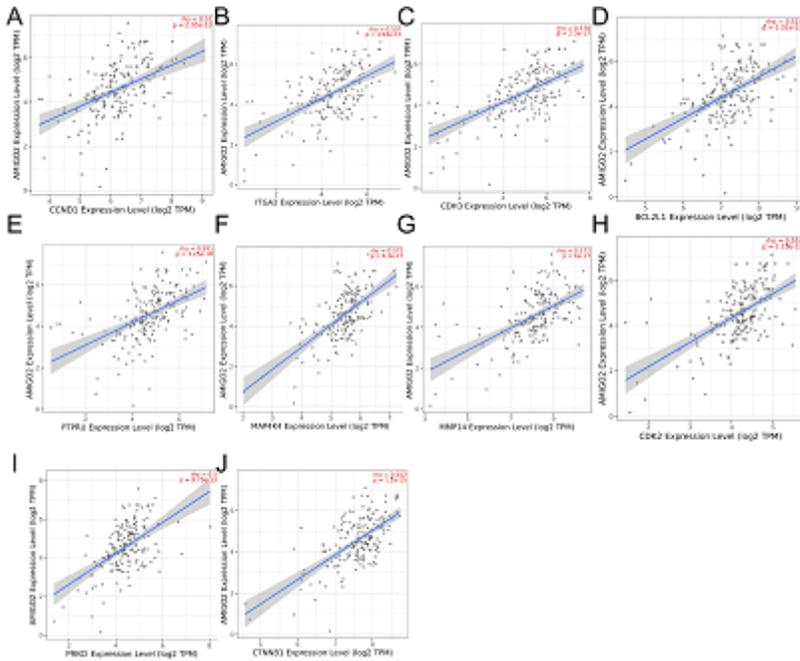


Figure 8

Ten genes positively correlated with AMIGO2 in pancreatic cancer in TIMER database (p value <0.05 Pearson correlation coefficient >0.5).

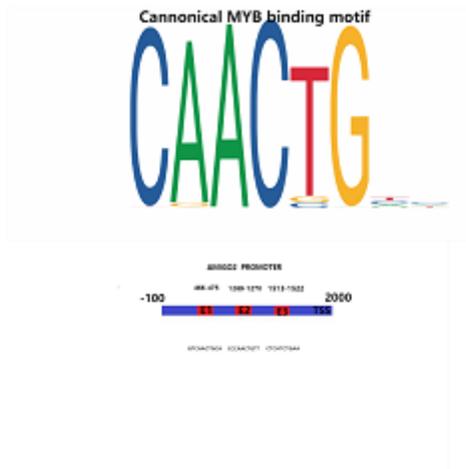


Figure 9

JASPAR site displays the three most MYB and AMIGO2 binding sites.

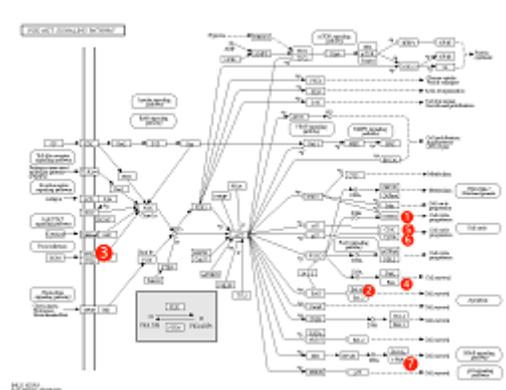


Figure 10

AMIGO2 plays a regulatory role in pancreatic cancer through PI3K-Akt signaling pathway.