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Integrative Bioinformatics Analysis Reveals Key Hub Genes and Pathways Driving Pancreatic Adenocarcinoma Progression

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ABSTRACT: Background

Pancreatic adenocarcinoma (PAAD) is one of malignant tumors with the highest fatality rate worldwide. This disease has several particularly intractable characteristics: most cases have progressed to an advanced stage when diagnosed, the condition progresses rapidly, and there are still very few effective clinical treatment options available at present. To identify new diagnostic markers and therapeutic targets, it is necessary to first comprehensively clarify the molecular mechanism underlying this cancer; otherwise, no new direction for diagnosis and treatment can be found.

Methods

Genes related to pancreatic cancer were recovered from GeneCards as well as CTD databases, moreover overlapping targets were subjected to KEGG pathway enrichment and protein–protein interaction (PPI) analysis using STRING as well as Cytoscape. Hub genes were recognized through CytoHubba & MCODE plug-ins. Their expression patterns were evaluated using UALCAN, GEPIA, OncoDB, and MuTarget, while TIMER and TISIDB were utilized to assess ICI and immunoregulatory correlations. MEXPRESS was applied to examine promoter methylation, and DGIdb was utilized to determine drug–gene interactions.

Results

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The study ultimately confirmed six hub genes: AKT1, TP53, EGFR, KRAS, MTOR, and STAT3, which are core factors regulating the development of pancreatic adenocarcinoma. Compared with normal tissue samples, these genes all showed significant abnormal expression in all tumor samples, and these abnormal levels of expression were also associated with tumor stage, grade, and the mutation status of the patient's own TP53 gene.

Subsequent survival analysis showed that the higher the expression levels of the four genes EGFR, KRAS, MTOR, and STAT3, the worse the patient's prognosis. Analysis of methylation and mutation status also proved that these genes are simultaneously controlled by the both genetic as well as epigenetic mechanisms, while the results of drug interaction analysis suggested that they can all be used as potential therapeutic targets for subsequent targeted drug development.

Conclusion

This comprehensive bioinformatics study identified six core hub genes that have diagnostic, prognostic, and therapeutic value for pancreatic adenocarcinoma. These results provide important support for understanding the molecular mechanism behind the progression of pancreatic adenocarcinoma, and also point out promising candidate directions for the subsequent development of precise diagnosis and treatment plans.

INTRODUCTION

Pancreatic cancer is currently the deadliest malignant tumor worldwide. It's often already in an advanced level when diagnosed, progresses rapidly, and most existing treatment methods are rarely effective. More specifically, the vast majority of the pancreatic cancers grow in the exocrine region of pancreas—part of pancreas responsible for secreting substances related to digestion.

The "pancreatic cancer" that people commonly refer to in daily life is in most cases specifically pancreatic adenocarcinoma, namely PAAD, which accounts for the vast majority of all diagnosed pancreatic malignancies. Although there are other rare subtypes of pancreatic cancer besides this most common type, when all pancreatic malignancies are taken into account, more than 95% occur in the exocrine part of the pancreas, specifically developing from acinar cells moreover ductal cells within the exocrine region (Bengtsson et al., 2020; Sarantis et al., 2020; Vareedayah et al., 2018).

Even though clinical treatment methods have seen many improvements in some years, the five-year of survival rate of pancreatic cancer patients remains shockingly low. The core reason for this situation has always been the lack of reliable, effective biomarkers—simply put, biomarkers are special signals in the body that can reflect the presence of disease. Without these signals, it is impossible to detect lesions in the early stages of cancer, nor to identify therapeutic targets that can act precisely (Bengtsson et al., 2020; Jafari et al., 2022).

To find pancreatic cancer at an early level when a cure is still possible, there are two insurmountable hurdles. The first hurdle is that cancer do not cause any obvious discomfort in its early levels, so patients cannot detect any abnormalities on their own. The second hurdle is still the problem of biomarkers: without biomarkers that can be used for convenient screening, it is impossible to identify issues before the condition worsens (Kaur et al., 2012).

Current routine diagnostic methods commonly used in hospitals rarely can detect tumors when radical treatment is still possible, and this predicament has made the need for new molecular biomarkers increasingly urgent—these are molecular signals in the body that are similar to the aforementioned biomarkers and can reflect disease status. Only with such biomarkers can we achieve early diagnosis, predict the subsequent course of disease in advance, and support the formulation of personalized treatment plans truly suitable for each patient (Jafari et al., 2022; Kaur et al., 2012).

In some years, bioinformatics has gradually become one of the most powerful tools for solving complex molecular-level problems of cancer. Its task is to integrate massive amounts of genomic and transcriptomic data and conduct systematic analysis. Through this approach, we can systematically identify all key nodes that promote the occurrence and development of tumors—clarifying which are core genes, which are related physiological pathways, and what interactions exist between molecules.

These computer-based analytical methods can also help us find the usable biomarkers that we have long lacked. Only by identifying these biomarkers can we gradually uncover the molecular mechanisms behind the occurrence as well as growth of the pancreatic cancer (Karimi et al., 2025; Qin & Zhao, 2014; Vandenbrouck et al., 2019).

These aspects are particularly closely associated with three factors: tumor progression, tumor metastasis, as well as the complete survival period of patients after diagnosis (Dong et al., 2018).

The pancreatic cancer we often refer to is mostly specifically pancreatic adenocarcinoma, namely PAAD, which is the general kind of cancer. The goal of this study is precisely to use bioinformatics methods to identify potential core genes in PAAD.

The study integrates a series of computer-based analytical methods, including PPI analysis, mRNA, core gene screening, and protein expression profile analysis, methylation status assessment, and mutation and druggability evaluation. It also analyzes the relations among these genes and ICI, related microRNAs, and transcription factors. The purpose of conducting all these analyses is to clarify the core molecular features and regulatory mechanisms that drive the progression of PAAD. Ultimately, this study can help people gain a deeper knowledge of the molecular characteristics that govern the progress of PAAD.

METHODOLOGY

Data collection and analysis

This study used two publicly accessible and queryable databases, namely GeneCards (https://www.genecards.org/) as well as CTD (https://ctdbase.org/), with the relevant citations marked as (Davis et al., 2025; Stelzer et al., 2016). To screen for genes associated with pancreatic cancer from these two databases, we uniformly used "Pancreatic ductal adenocarcinoma" as the keyword to search all datasets in both databases. After that, we also used the open-source tool InteractiveVenn (https://www.interactivenn.net/) to draw a Venn diagram, to identify the overlapping portion among the two gene sets, with the relevant citation marked as (Heberle et al., 2015). These common genes that appeared in both datasets were retained for use in all subsequent analyses.

KEGG enrichment analysis

In this study, we first screened a batch of genes related to our research topic. To clarify how critical these genes are in organisms and what specific functional tasks they undertake, we adopted KEGG pathway improvement analysis to sort out the relevant information of these genes.

All calculation processes of the entire analysis were completed via the ShinyGO 0.85 online web server, the access URL of which is https://bioinformatics.sdstate.edu/go/. It is a specialized online bioinformatics tool that can help us complete functional enrichment analysis of gene sets, and also produce intuitive and easy-to-understand visualizations of the analysis results. Relevant content about this tool was published by Ge et al., 2020.

The core goal of conducting this analysis is to identify all key signaling pathways and metabolic pathways related to the candidate genes determined in this study. After sorting out the associations between these genes and pathways, we can fully clarify the underlying molecular mechanism that drives the progression of PAAD.

PPI, Module analysis, and Hub genes identification

First, import the pre-screened genes into the STRING database (https://string-db.org/) to construct a Homo sapiens PPI network. During network construction, a self-assurance threshold is set for the interactions within the network, and only interactions with a score ≥ 0.4 are included (Szklarczyk et al., 2023). After the network is constructed, it is processed using version 3.10.3 of the Cytoscape software. First, this abstract network is converted into an intuitive visual map, and subsequent network analysis is carried out at the same time. Within Cytoscape, we used two dedicated plugins to complete different analysis tasks: first, the MCODE plugin was used to isolate gene clusters with particularly tight connections between genes from the entire large network; then, CytoHubba plugin was used to assess the reputation of each node in the network one by one through core centrality parameters including degree and closeness. Finally, those genes that were simultaneously marked by MCODE and CytoHubba are selected as core genes for all subsequent downstream analyses.

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mRNA and protein expression analysis of the identified hub genes in Pan cancer

Two publicly available bioinformatics tools to analyze the abnormalities of these core genes. The first tool is the TIMER database, with the access URL http://cistrome.org/TIMER/. This database completed the mRNA expression analysis of core genes across pan-cancer types, and the relevant findings were published by Li et al., 2017. The second tool is the Human Protein Atlas, abbreviated as HPA, with its URL https://www.proteinatlas.org/. This tool assessed the appearance of proteins encoded by the same batch of core genes analyzed by TIMER in numerous kinds of cancer, as well as the relevant results were released by Digre & Lindskog, 2021. After summarizing the two sets of data, it was found that these genes all exhibited abnormal changes at the translational and transcriptional levels in PAAD and other malignant tumors.

Validating the expression Profile of the Hub genes

UALCAN database (https://ualcan.path.uab.edu/, Chandrashekar et al., 2022) to analyze the mRNA expression of hub genes in PAAD. During the analysis, we incorporated multiple clinical parameters related to the patients' conditions and disease status, specifically including sample type, cancer stage, tumor grade, TP53 mutation, and chronic pancreatitis. To confirm the dependability of the outcomes obtained from the UALCAN database, we then used three additional online tools for further validation, namely OncoDB (https://oncodb.org/), GEPIA (http://gepia.cancer-pku.cn/), as well as MuTarget (https://www.mutarget.com/), with the corresponding citations for the three tools being Tang et al., 2017; Nagy & Győrffy, 2021; Cho et al., 2025. Finally, we visualized the gene expression differences obtained from these analyses in the form of box plots for intuitive presentation.

Correlation analysis

The association analysis of identified hub genes was completed utilizing the OncoDB database (https://oncodb.org/)(Cho et al., 2025). Correlation analysis was conducted to assess the relationships between the hub genes. Correlations were measured using the Pearson association coefficient, as well as a p-value of below 0.05 was measured statistically important.

Prognostic analysis of hub genes

To evaluate the prognostic relevance of the identified hub genes, the HPA (https://www.proteinatlas.org/) database was utilized to assess their levels of protein expression in PAAD tissues.(Digre & Lindskog, 2021). The HPA database offers immunohistochemistry (IHC)-based protein expression profiles across different human tissues, which allows the visualization and comparison of the staining intensity, localization, and distribution of the target proteins. Such a study helps to comprehend the link among the protein expression patterns and the clinical outocmes of pancreatic cancer patients.

Immune cell infiltration and correlation analysis

To analyse the relationship among hub gene expression and ICI in PAAD, the TIMER web server (http://cistrome.org/TIMER/) was utilized (Li et al., 2017). We estimated the plenty of various types of immune cell, including B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, and neutrophils, using TIMER. Spearman's rank association was used to evaluate the correlation among hub gene expression and the level of immune infiltration.

Survival analysis

Kaplan–Meier Plotter (KM Plotter) (https://kmplot.com/analysis/) database was utilized to assess the prognostic significance of the identified hub genes in PAAD (Posta & Győrffy, 2025). OS was analyzed to evaluate the prognostic significance of the identified hub genes.

Mutation analysis

Using the cBioPortal database (https://www.cbioportal.org/)(de Bruijn et al., 2023), the mutation rates of the hub genes identified in PAAD were analyzed. The "Pancreatic Adenocarcinoma (QCMG, Nature 2016)" dataset, which includes 456 samples, was chosen as the source of data. The occurrence and kinds of genetic alterations, such as mutations and copy number variations, in the selected hub genes were investigated to better understand their genomic variation patterns in pancreatic cancer better.

Methylation level expression analysis

The MEXPRESS tool (https://mexpress.ugent.be/) was utilized to assess DNA promoter methylation stages of the recognized hub genes in PAAD patients (Koch et al., 2015).

MEXPRESS is an interactive online platform that compiles TCGA data and thus enables the visualization of the links between gene promoter methylation changes and clinical parameters. This analysis provided visions into the potential epigenetic rule of the hub genes in pancreatic cancer.

Druggability analysis

The DGIdb (https://dgidb.org/) was used to evaluate the druggability possibility of the identified hub genes. DGIdb is an integrated resource that aggregates data on drug–gene connections and possible druggable targets from multiple curated databases as well as published literature (Cannon et al., 2024). The analysis further determines the therapeutic target potential of the identified hub genes.

Association between hub genes, Transcription factors, and miRNAs.

To identify potential regulatory TFs related with identified hub genes, the Enrichr platform (https://maayanlab.cloud/Enrichr/) was employed (Chen et al., 2013). The analysis utilized the JASPAR and TRANSFAC position-specific weight matrix (PWM) databases to estimate TF–gene interactions. Similarly, to explore microRNAs (miRNAs) potentially regulating hub genes, miRTarBase database integrated within Enrichr was utilized. These analyses aimed to uncover key transcriptional and post-transcriptional regulators influencing the expression of hub genes in PAAD.

Result and Discussion

Identification and Collection of PAAD –Associated Genes

A total of 6,811 genes were retrieved from GeneCards, while 12,464 genes were obtained from CTD. After compiling and comparing the datasets, a total of 19,275 unique genes related to PAAD were identified. A Venn diagram was constructed to determine the overlay among the two datasets, revealing 4,461 common genes that were shared by both databases (Figure 1a). These overlapping genes were identified as high-confidence pancreatic cancer–associated genes and were nominated for additional bioinformatics analysis.

KEGG Pathway Enrichment

The 4,461 overlapping genes were imperiled to the KEGG pathway improvement analysis utilizing the ShinyGO 0.85 web tool to explore their biological significance. The analysis exposed that a considerable number of genes were meaningfully enriched in cancer-related signaling pathways. Notably, 67 genes were supplemented in "Pancreatic cancer (hsa05212)" pathway, exhibiting a highly significant false discovery rate (FDR) of 1.10E-38, indicating a strong association with pancreatic carcinogenesis.

Along with the PCP, a number of other important oncogenic pathways have also been found to be highly enriched, such as Pathways in Cancer, PI3K-Akt signaling pathway, as well as Proteoglycans in Cancer, and all these pathways are recognized to play an important role in cancer. The bar plot illustrating the top enriched pathways is shown in Figure 1b, while Figure 1c depicts the detailed KEGG "Pancreatic cancer" pathway generated by ShinyGO.

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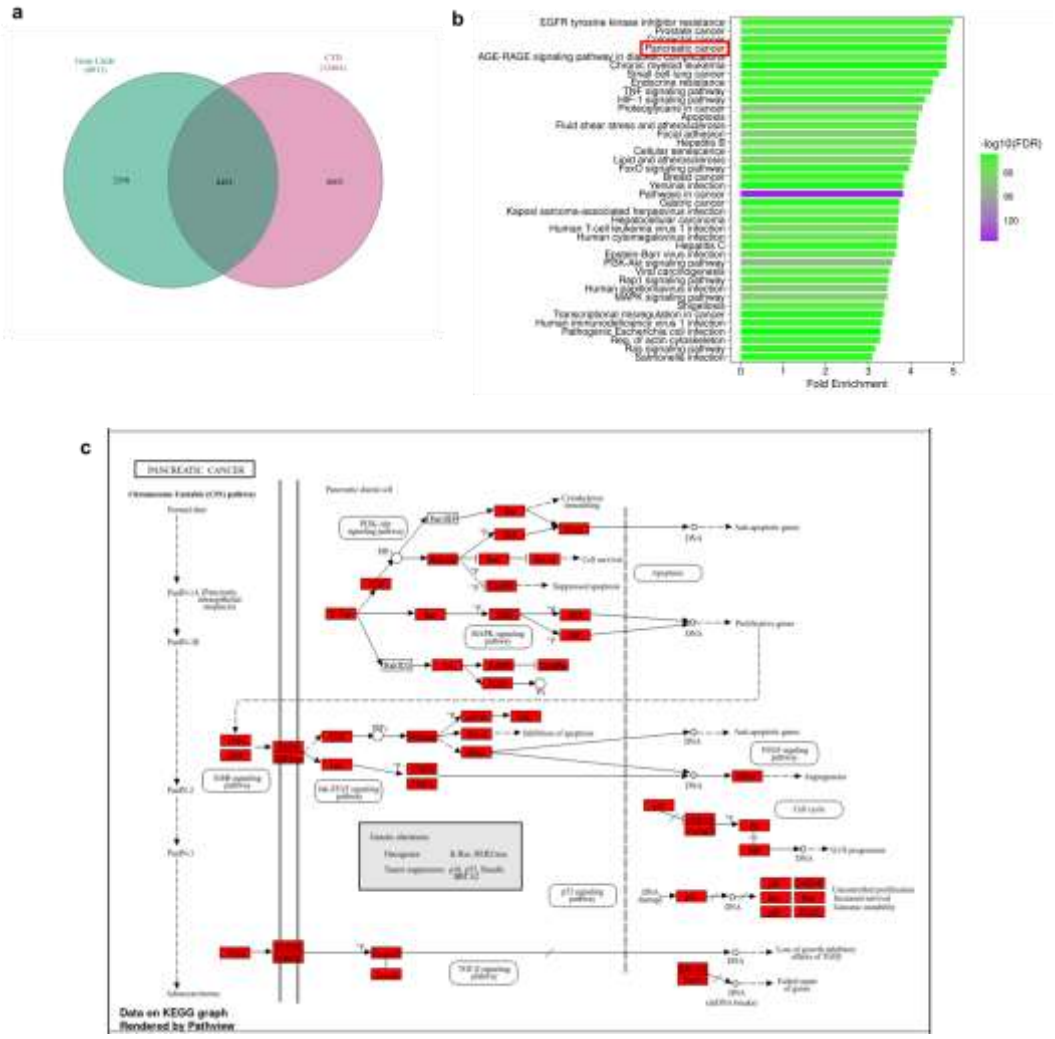

Figure 1. (a) Diagram that shows the common target genes of pancreatic cancer from GeneCards and CTD sources. (b) KEGG pathway enrichment barplot of overlapped genes from ShinyGO (c) KEGG pathway map of the Pancreatic cancer as identified through enrichment analysis.

PPI Network Construction, Module Detection, and Hub Genes Identification

A total of 67 genes were found to be associated with the pancreatic cancer pathway and analyzed for PPI network analysis with the help of STRING database (Figure 2a). The network obtained from the analysis showed a significantly high PPI enrichment p-value $< 1.0 \times 10^{-16}$, suggesting that the interactions were not accidental. The network comprised 67 nodes connected by 951 edges, suggesting extensive functional associations among the genes. PPI network obtained from STRING was imported as well as visualized in Cytoscape (Figure 2b).

To identify highly interconnected clusters, the MCODE plug-in in Cytoscape 3.10.3 was employed, which detected four distinct modules (Table 1). Among them, Module 1 (Figure 2c) exhibited the highest score of 29.5, consisting of 33 nodes and 472 edges, followed by Module 2 (Figure 2d) (score = 3.33; 4 nodes, 5 edges), Module 3 (score = 3; 3 nodes, 3 edges) (Figure 2e), and Module 4 (score = 2.67; 4 nodes, 4 edges).

Further, CytoHubba analysis was accomplished to classify top-ranked genes based on degree and closeness centrality (Table 2). The intersection of both ranking methods yielded six hub genes: AKT1, TP53, EGFR, KRAS, MTOR, and STAT3. Notably, MTOR ranked fifth by degree and STAT3 ranked fifth by closeness, leading to their inclusion as key nodes. Importantly, all six

of these hub genes were located within Module 1, representing their strong interconnectivity and biological significance within the pancreatic cancer.

Table 1. MCODE-identified modules within the PPI network.

Cluster	Score (Density*Nodes)	Nodes	Edges	Node IDs
1	29.5	33	472	SMAD3, PIK3CA, TGFB1, BRAF, CASP9, MAPK1, CDKN2A, MAP2K1, MAPK3, RAF1, EGFR, SMAD2, CDKN1A, MTOR, CDK4, RELA, STAT1, ERBB2, KRAS, MAPK8, TP53, JAK1, CCND1, AKT2, NFKB1, E2F1, EGF, RPS6KB1, SMAD4, AKT1, STAT3, PIK3R1, BCL2L1
2	3.333	4	5	RAC1, PIK3CD, PIK3R3, PIK3R2
3	3	3	3	E2F2, E2F3, RB1
4	2.667	4	4	CDK6, TGFBR2, BRCA2, PIK3CB

Table 2. Top-ranked hub genes were recognized based on degree as well as closeness importance using CytoHubba.

Rank	Name	Degree Score	Closeness Score
1	AKT1	58	62
2	TP53	56	61
3	EGFR	53	59.5
4	KRAS	51	58.5
5	MTOR	49	-
6	STAT3	-	57.5

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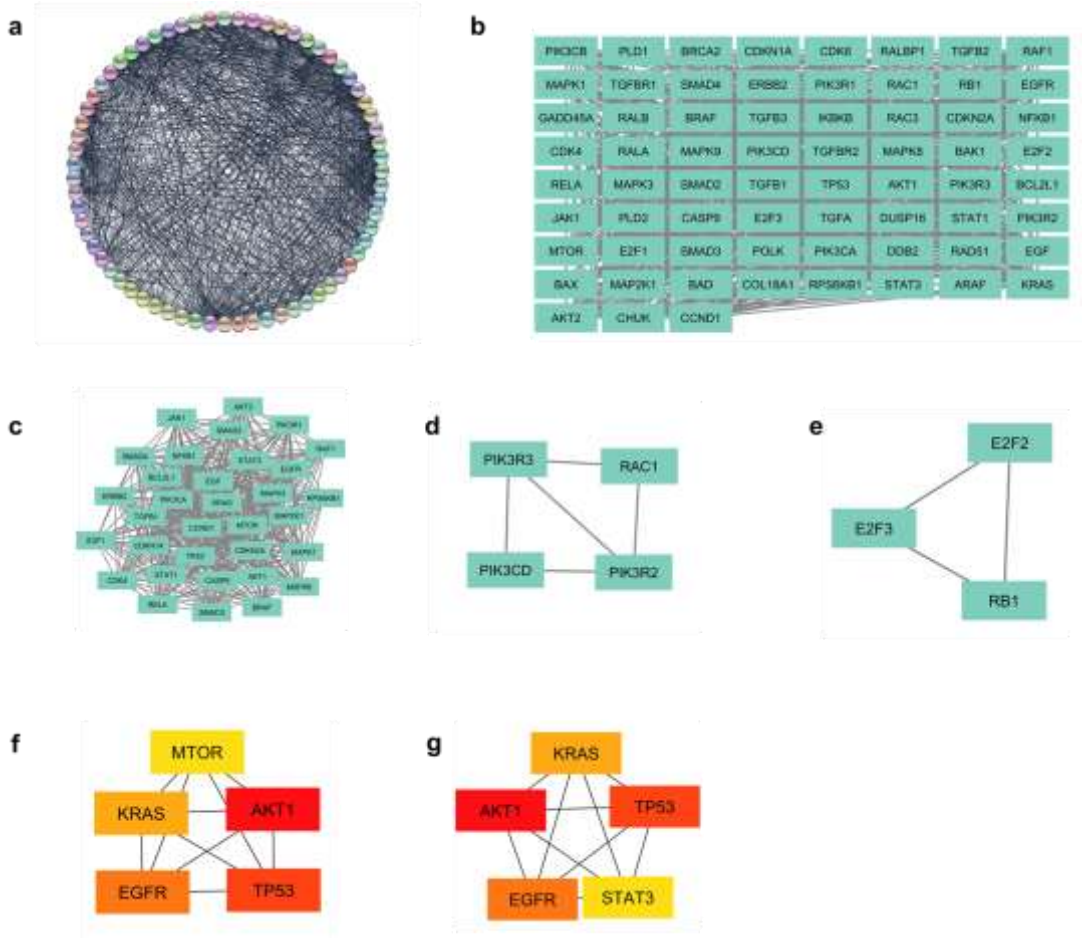

Figure 2 . a) PPI network from the STRING database; (b) visualization of the constructed PPI network using Cytoscape; (c-e) the three significant modules identified using the MCODE plug-in: (c) Module 1 (score 29.5), (d) Module 2 (score 3.3), and (e) Module 3 (score 3); Cytohubba analysis showing the top-5 ranked genes based on (f)degree and (h) closeness

mRNA and Protein Expression Patterns of Hub Genes Across Pan-Cancer Datasets

The protein expression profiles of the six identified hub genes—AKT1, TP53, EGFR, KRAS, MTOR, and STAT3—were examined across various cancer types using the HPA database. all six genes exhibited protein expression in pancreatic cancer tissues(Figure 3a). These outcomes recommend that the recognized hub genes are consistently overexpressed or highly active in pancreatic cancer, underscoring their probability as a diagnostic biomarkers.

The mRNA expression levels of hub genes were analyzed across numerous cancer types using the TIMER database. The analysis revealed that all six hub genes exhibited variable expression patterns across different cancers, including PAAD (Figure 3b). Notably, each of these genes showed suggestively elevated transcript levels in the PAAD compared to corresponding normal tissues, indicating their potential involvement in pancreatic tumorigenesis.

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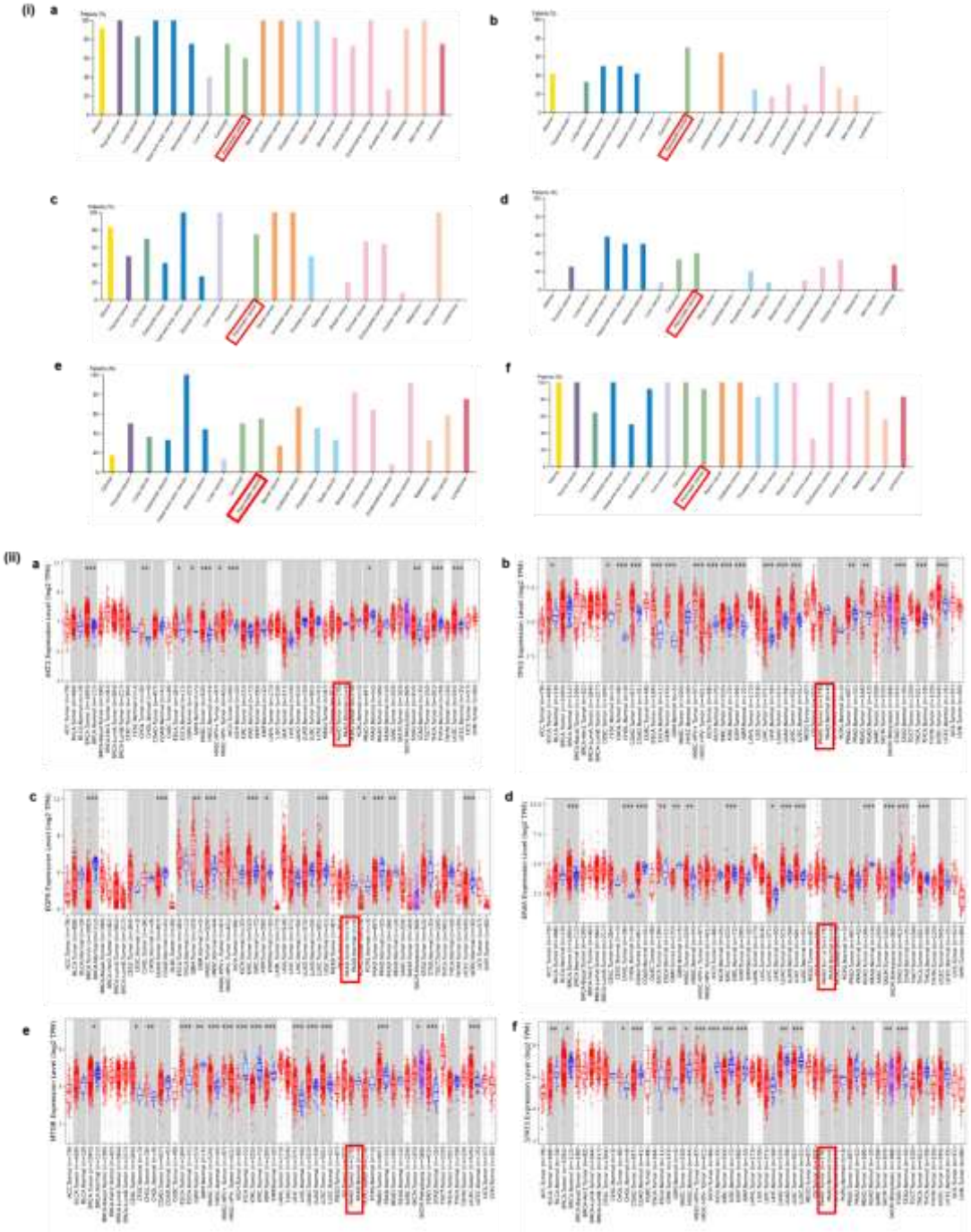

Figure 3. mRNA expression points of the identified hub genes in PAAD. (i) Protein Atlas analysis showing the appearance of (a) AKT1, (b) TP53, (c) EGFR, (d) KRAS, (e) mTOR, and (f) STAT3 proteins across various cancer types using the Human Protein Atlas database. (ii)Pan Cancer Analysis of (a) AKT1 (b)TP53 (c) EGFR (d) KRAS (e) mTOR (f)STAT3 mRNA expression by using the TIMER database.

Validation of Hub Genes Expression in PAAD

To further validate the expression profile of the discovered hub genes, the mRNA expression level of these genes was analyzed in pancreatic adenocarcinoma (PAAD) tissues and normal pancreatic tissues through the UALCAN database. The result showed the differences in the expression profile of these six hub genes between tumors and normal tissues.

The results revealed that AKT1, EGFR, KRAS, and STAT3 were suggestively upregulated in PAAD tissues associated to normal controls, whereas TP53 and MTOR were notably downregulated (Figure 4b). The heatmap (Figure 4a) clearly illustrates these contrasting expression patterns between tumor and normal samples.

When analyzed according to individual cancer stages, the expression points of hub genes AKT1, KRAS, MTOR, and STAT3 showed a progressive increase from early to advanced stages of PAAD. In contrast, TP53 and EGFR exhibited variable expression patterns across stages, with relatively lower expression in early stages and elevated levels in later stages (Figure 4c). These consequences recommend that appearance of several hub genes correlates with disease advancement, indicating their potential role in pancreatic cancer progression.

Based on tumor grade, EGFR and KRAS showed a gradual increase in expression from grade 1 to grade 3, followed by a slight decrease at grade 4. STAT3 expression decreased with increasing tumor grade, while AKT1 and MTOR showed variable expression without a clear trend. TP53 expression was lower in grades 1–3 and relatively higher in grade 4 (Figure 4d).

Based on TP53 mutation status, the analysis revealed that AKT1, EGFR, and KRAS were upregulated in TP53-mutant PAAD samples as compared to the normal tissues. In contrast, TP53, MTOR, and STAT3 showed lower expression levels in TP53-mutant and non-mutant samples relative to normal controls (Figure 4e). Among the upregulated genes, AKT1, EGFR, and KRAS and exhibited slightly higher in expression in TP53-mutant compared to non-mutant samples, suggesting that TP53 mutations might subsidize to the activation of oncogenic signaling pathways.

In contrast, MTOR expression was slightly higher in patients with pancreatitis compared to those without pancreatitis, while STAT3 expression was notably elevated in this group (Figure 4f).

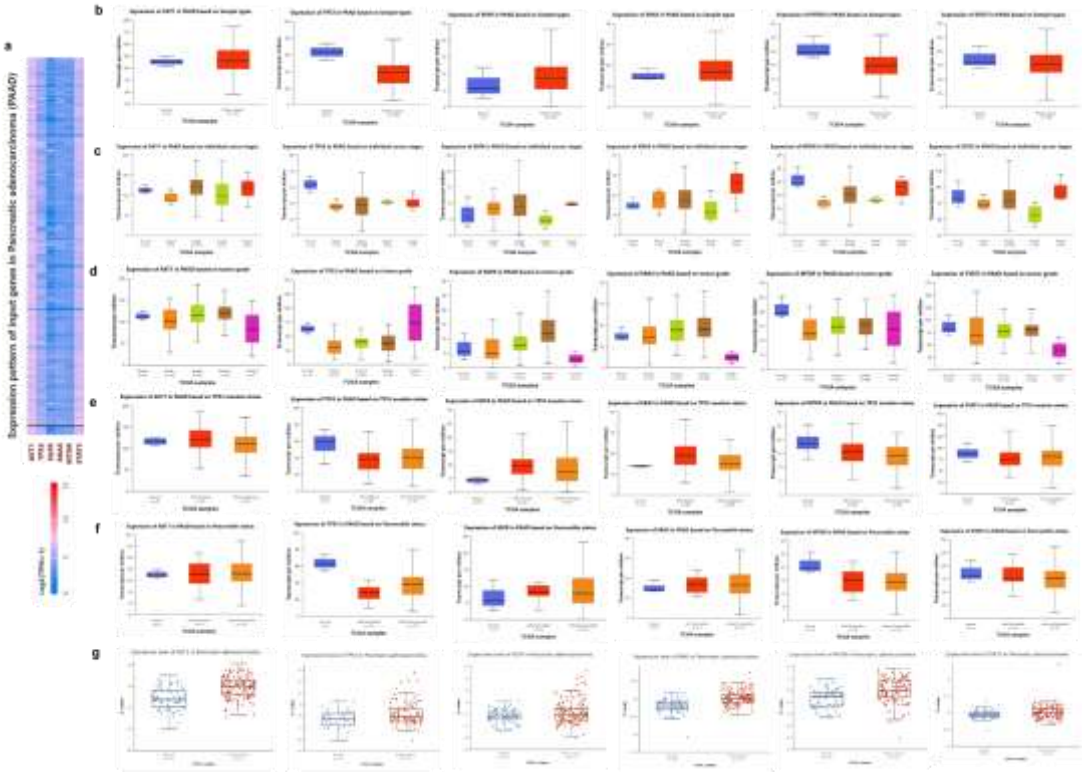

Figure 4. (a) A heatmap of AKT1, TP53, EGFR, KRAS, mTOR, and STAT3 in the PAAD sample group and the normal control group. Clinicopathological features of PAAD associated with the mRNA expression of AKT1, TP53, EGFR, KRAS, mTOR, and STAT3 obtained from UALCAN (b) contrast of expression among PAAD and normal samples; (c) expression analysis across different cancer stages; (d) expression patterns across various tumor grades; (e) correlation among TP53 mutation status and gene expression; (f) association of gene expression with chronic pancreatitis (g) comparison of protein expression among PAAD as well as normal tissues.

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Similarly, the total protein expression points of the identified hub genes were analyzed in PAAD as well as normal pancreatic tissues using UALCAN (CPTAC dataset). The jitter plot analysis revealed that primary PAAD tumor samples exhibited significantly higher protein expression levels of AKT1, EGFR, KRAS, MTOR, TP53, and STAT3 compared to the normal tissues, representative their potential involvement in pancreatic tumor progression (Figure 4g).

According to GEPIA analysis, all six hub genes were suggestively upregulated in PAAD tissues relative to normal controls (Figure 5(i)).

This consistent overexpression of all hub genes indicates their probability involvement in pancreatic tumor growth as well as progression (Figure 5(ii)).

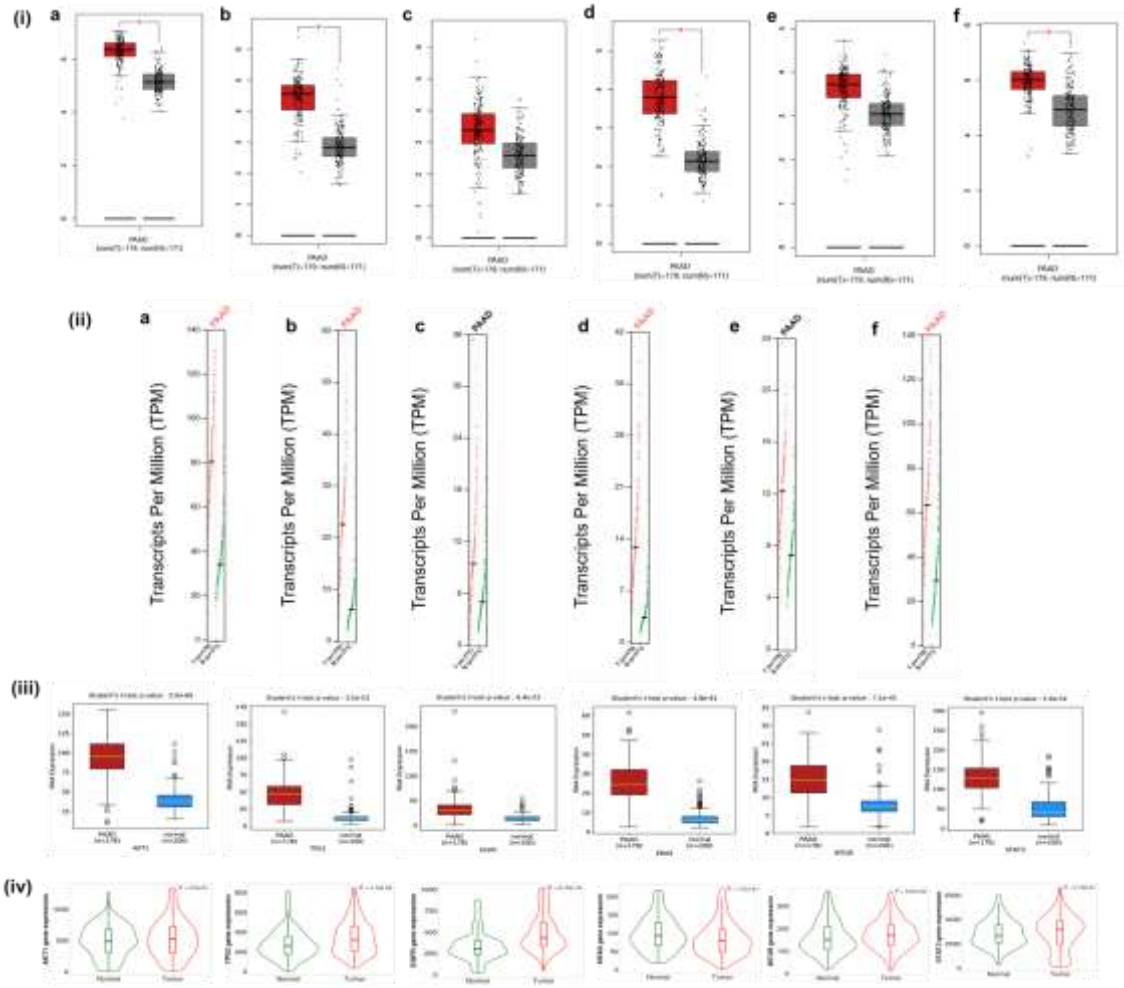

Figure 5. Clinical correlation and expression validation analysis of the hub genes (a) AKT1 (b) TP53 (c)EGFR (d)KRAS (e)mTOR (f) STAT3 in PAAD using multiple databases: (i) mRNA look hub genes levels from GEPIA, where red signifies tumor samples as well as grey signifies normal samples; (ii) copy number difference of hub genes from GEPIA, with red indicating tumor samples and green indicating normal samples; (iii) expression validation of the hub genes in PAAD as well as normal tissues utilizing the OncoDB database; (iv) expression validation of the hub genes in PAAD moreover normal tissues utilzing the MuTarget database.

Consistently, analysis using the OncoDB database revealed statistically elevated mRNA expression levels of hub genes in pancreatic tumor samples comparative to normal controls. (Figure 5(iii)). Analysis using the MuTarget database revealed that AKT1, TP53, EGFR, MTOR, and STAT3 showed elevated mRNA expression levels in pancreatic tumor tissues associated to normal samples, with most differences being statistically significant (Figure 5(iv)).

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Correlation Analysis Among Hub Genes

The correlation analysis between the six identified hub genes was performed using the OncoDB database to assess their co-expression patterns in PAAD (Figure 6). Notably, AKT1 showed strong positive associations with EGFR (r = 0.60), KRAS (r = 0.70), MTOR (r = 0.70), and STAT3 (r = 0.60), whereas exhibiting a weaker correlation with TP53 (r = 0.20). Similarly, EGFR displayed significant connections with KRAS (r = 0.62), MTOR (r = 0.56), and STAT3 (r = 0.60). The correlations between KRAS–MTOR (r = 0.61) and KRAS–STAT3 (r = 0.67) were particularly notable, highlighting their cooperative roles in oncogenic signaling pathways. MTOR showed a moderate association with STAT3 (r = 0.70). In contrast, TP53 exhibited relatively weaker but significant associations with EGFR (r = 0.27), KRAS (r = 0.22), MTOR (r = 0.32), and STAT3 (r = 0.30). All observed correlations were statistically significant, indicating a coordinated expression pattern among these hub genes in PAAD.

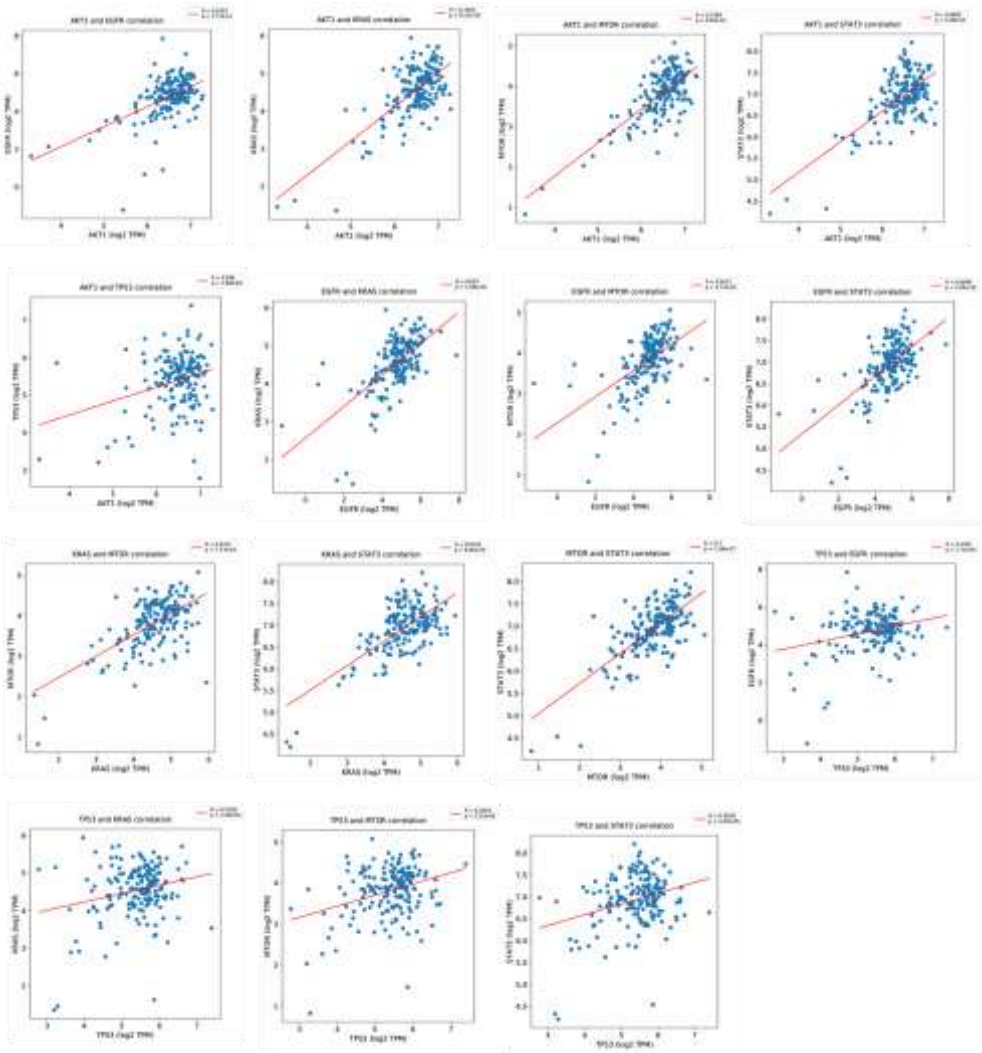

Figure 6. Correlation analysis of the identified hub genes using the OncoDB database.

Prognostic Meaning of Hub Genes in PAAD

Immunohistochemistry (IHC) data from patient tissue samples revealed that AKT1, TP53, EGFR, and MTOR exhibited high staining intensity, indicating strong protein expression in pancreatic cancer tissues. In contrast, KRAS and STAT3 showed moderate staining intensity, corresponding to medium protein expression levels (Figure 7). These findings confirm the elevated expression of hub genes at the level of protein, supporting their potential prognostic and functional significance in PAAD.

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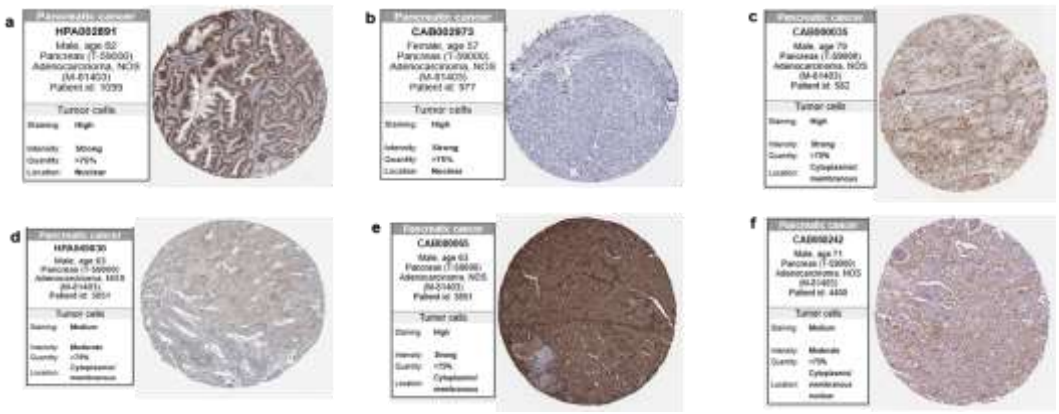

Figure 7. Immunohistochemistry analysis of the hub genes expression in pancreatic cancer tissues gotten from Human Protein Atlas database.

Immune Cell Infiltration & Correlation of the Hub Gene Expression

Subsequently, the relations among ICI (CD8+ T cells, CD4+ T cells, B cells, neutrophils, and macrophages) moreover expression of the hub genes in PAAD samples. As shown in Figure 8, most hub genes displayed positive correlations with multiple immune cell types, signifying their possible part in modulating the tumor immune microenvironment. Specifically, AKT1, TP53, EGFR, KRAS, MTOR, and STAT3 exhibited important positive associations with infiltration levels of $CD8^+$ T cells, B cells, neutrophils, as well as macrophages, indicating that their elevated expression may subsidize to recruitment or activation of these immune cells in tumor milieu.

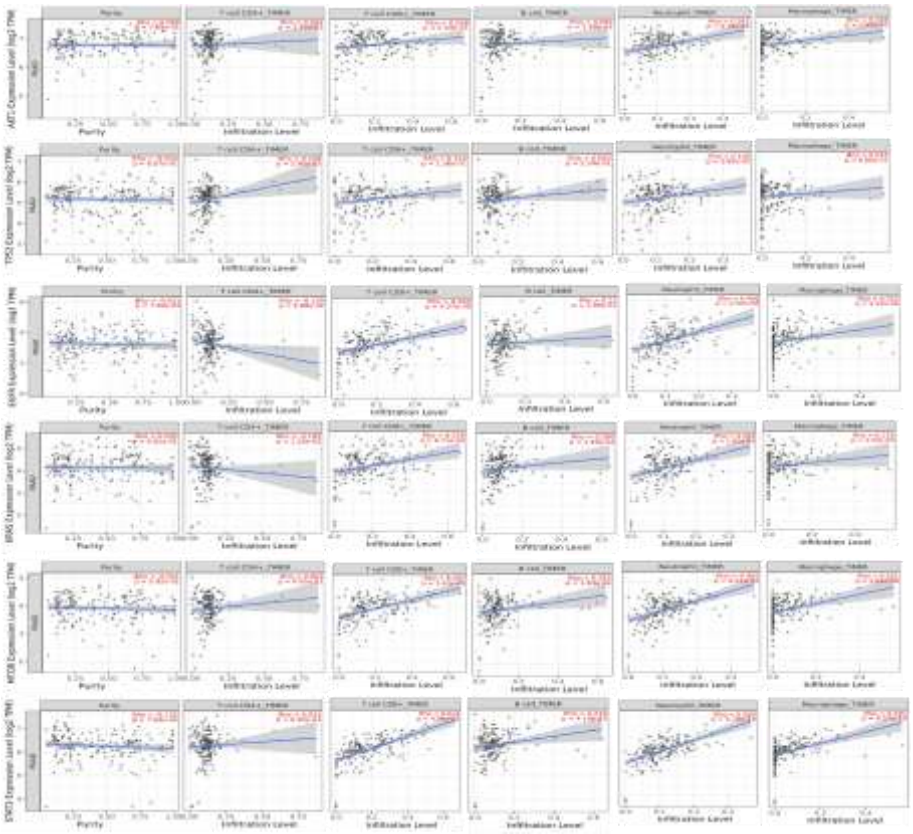

Figure 8. Correlation between hub genes expression as well as tumor-infiltrating immune cells in PAAD, as analyzed using the TIMER database.

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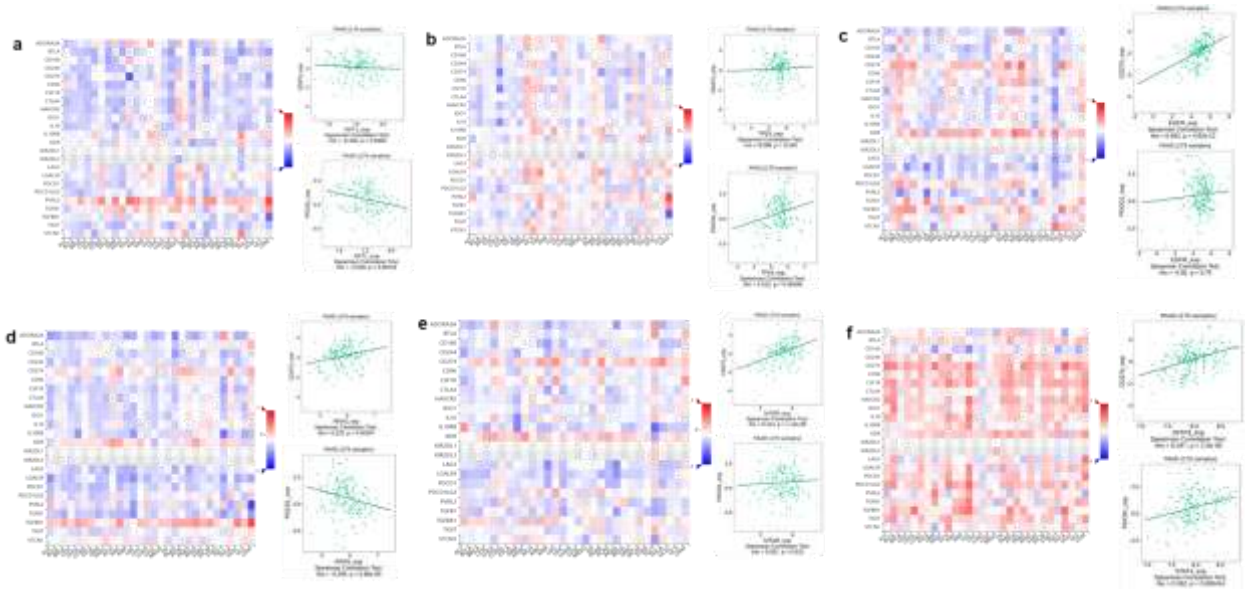

Figure 9. Expression of the (a) AKT1 (b) TP53 (c)EGFR (d)KRAS (e)mTOR (f) STAT3 in PAAD from the TISIDB database in relation to immunosuppression. Heatmap showing correlation among immunosuppressive molecules and the hub genes across numerous cancer types

The relationship among hub gene expression and immunosuppressive molecules — CD274 (PD-L1) and PDCD1 (PD-1) — was analyzed using the TISIDB database to assess the possible participation of these genes in immune regulation within PAAD (Figure 9).

The analysis revealed that AKT1 expression showed an negative association with both CD274 (rho = –0.156, p = 0.0366) and PDCD1 (rho = –0.239, p = 0.00131). TP53 exhibited a weak positive association with CD274 (rho = 0.088, p = 0.243) and a significant positive association with PDCD1 (rho = 0.222, p = 0.00286). EGFR showed a strong positive correlation with CD274 (rho = 0.483, p = 4.82e-12), whereas its association with PDCD1 was not significant (rho = –0.02, p = 0.79). Similarly, KRAS expression correlated positively with CD274 (rho = 0.222, p = 0.00287) but negatively with PDCD1 (rho = –0.294, p = 6.86e-05). MTOR expression demonstrated a strong positive association with CD274 (rho = 0.414, p = 1.13e-08) and no significant relationship with PDCD1 (rho = 0.037, p = 0.622). Finally, STAT3 exhibited significant positive correlations with both CD274 (rho = 0.347, p = 2.3e-06) and PDCD1 (rho = 0.262, p = 0.000414). The correlation heatmap analysis across multiple cancer types exposed that hub genes are closely associated with the expression of several immunosuppressive molecules.

Survival analysis

The prognosis significance of the six hub genes discovered in PAAD was assessed based on the information in KM Plotter database. As seen from Figure 10(i), differences in overall survival (OS) have been noticed in relation to the different expression levels of the genes.

Higher EGFR (HR = 1.31, 95% CI = 1.13-1.52, p = 2.68 x 10^{-4}), KRAS (HR = 1.24, 95% CI = 1.07-1.43, p = 0.00329), MTOR (HR = 1.16, 95% CI = 1.00-1.33, p = 0.0496), and STAT3 (HR = 1.26, 95% CI = 1.08-1.47, p = 0.00259) expressions indicated shorter overall survival of patients compared to those with low expression levels, suggesting their potential negative effect on the prognosis of PAAD. The high expression levels of these genes have been found to be significantly associated with the high hazard ratio (HR), indicating their possible connection to a poor prognosis and aggressive progression of PAAD.

Conversely, higher AKT1 expression was associated with good prognosis (HR = 0.83, 95% CI = 0.71-0.97, p = 0.0167), whereas the effect of the TP53 gene expression level on patient prognosis was insignificant (HR = 1.13, 95.

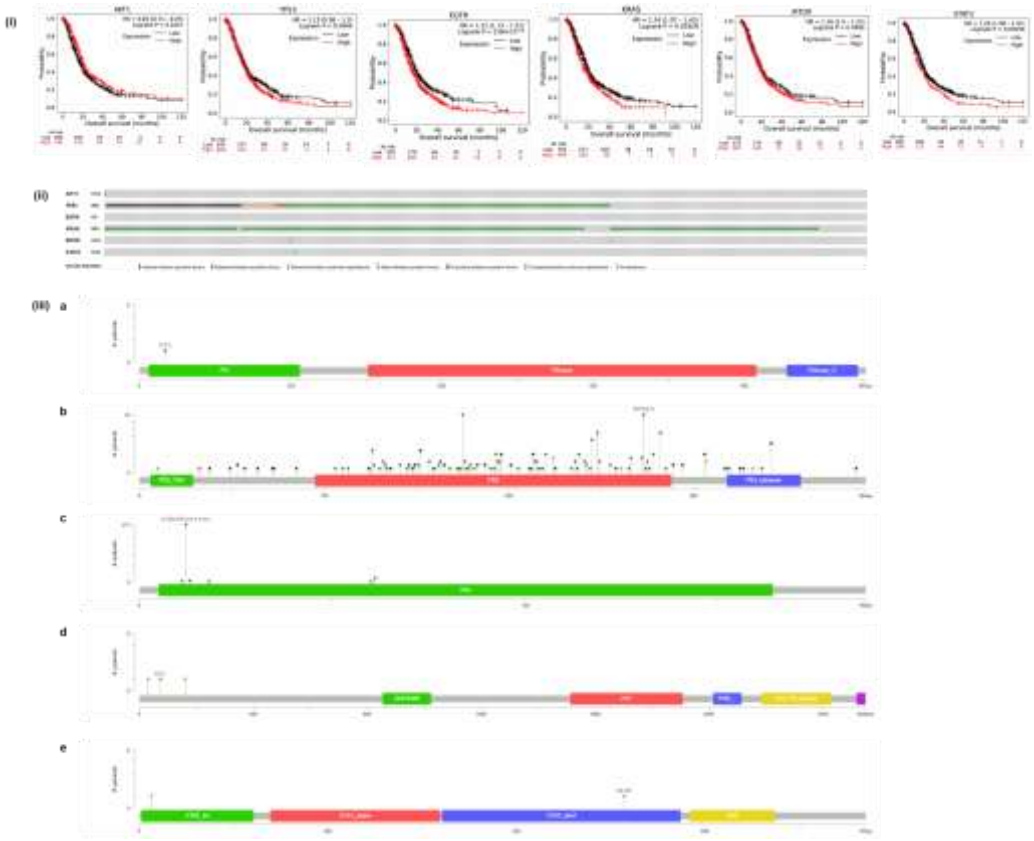

Figure 10. (i) Survival analysis of hub genes

(ii) The frequency of genetic alterations of the hub genes found in PAAD using the cBioPortal

(iii) The frequency and location of the genetic alterations in (a) AKT1, (b) TP53 (c) KRAS (d) mTOR (e) STAT3 in PAAD

Mutation Profiling of Hub Genes

Using the cBioPortal database, mutation profiling of the six hub genes was performed across 456 PAAD samples (Figure 10(ii)). Among these, the KRAS gene exhibited maximum frequency of genetic alterations (90%), followed by TP53 (66%), MTOR (0.8%), STAT3 (0.5%), and AKT1 (0.2%). Notably, EGFR showed no detectable mutations in the analyzed cohort. The identified genetic alterations encompassed a variety of mutation types, including missense mutations, in-frame mutations, splice site mutations, and truncating mutations, indicating the genetic heterogeneity of PAAD. Figure 10(iii) illustrates the distribution and location of these mutations within each hub gene.

Promoter Methylation Status

To assess the possible effect of promoter methylation on dysregulation of expression of hub genes in PAAD, we have assessed the correlation between their mRNA expression levels and promoter methylation profiles through the use of MEXPRESS database (Figure 11). This was done to evaluate if promoter methylation affects the expression of these hub genes in PAAD.

It is worth noting that our analysis showed different promoter methylation profiles of these hub genes in TCGA pancreatic adenocarcinoma (PAAD). AKT1 displayed weak-to-moderate promoter methylation, where a mild inverse correlation between methylation as well as expression suggested that methylation modestly suppressed transcription, while copy-number gain served as the primary contributor to its elevated expression. In contrast, TP53 exhibited no significant promoter methylation, indicating that its expression was largely independent of methylation status, with minimal influence from copy-number variation.

Similarly, EGFR showed negligible promoter methylation, and its increased expression was primarily associated with copy-number amplification, indicating transcriptional activation rather than epigenetic regulation. KRAS demonstrated promoter

methylation with significant negative correlations to gene expression, consistent with partial transcriptional repression; however, copy-number gain and mutational activation were identified as dominant mechanisms driving its overexpression.

For MTOR, partial promoter methylation was observed, with several CpG sites showing inverse associations with expression, though copy-number gain remained the major determinant of its transcriptional upregulation. Finally, STAT3 exhibited strong promoter methylation correlations, where hypermethylation reduced expression and hypomethylation enhanced transcription, indicating that the epigenetic mechanisms play a critical function in modifiable its expression, complemented by upstream oncogenic signaling

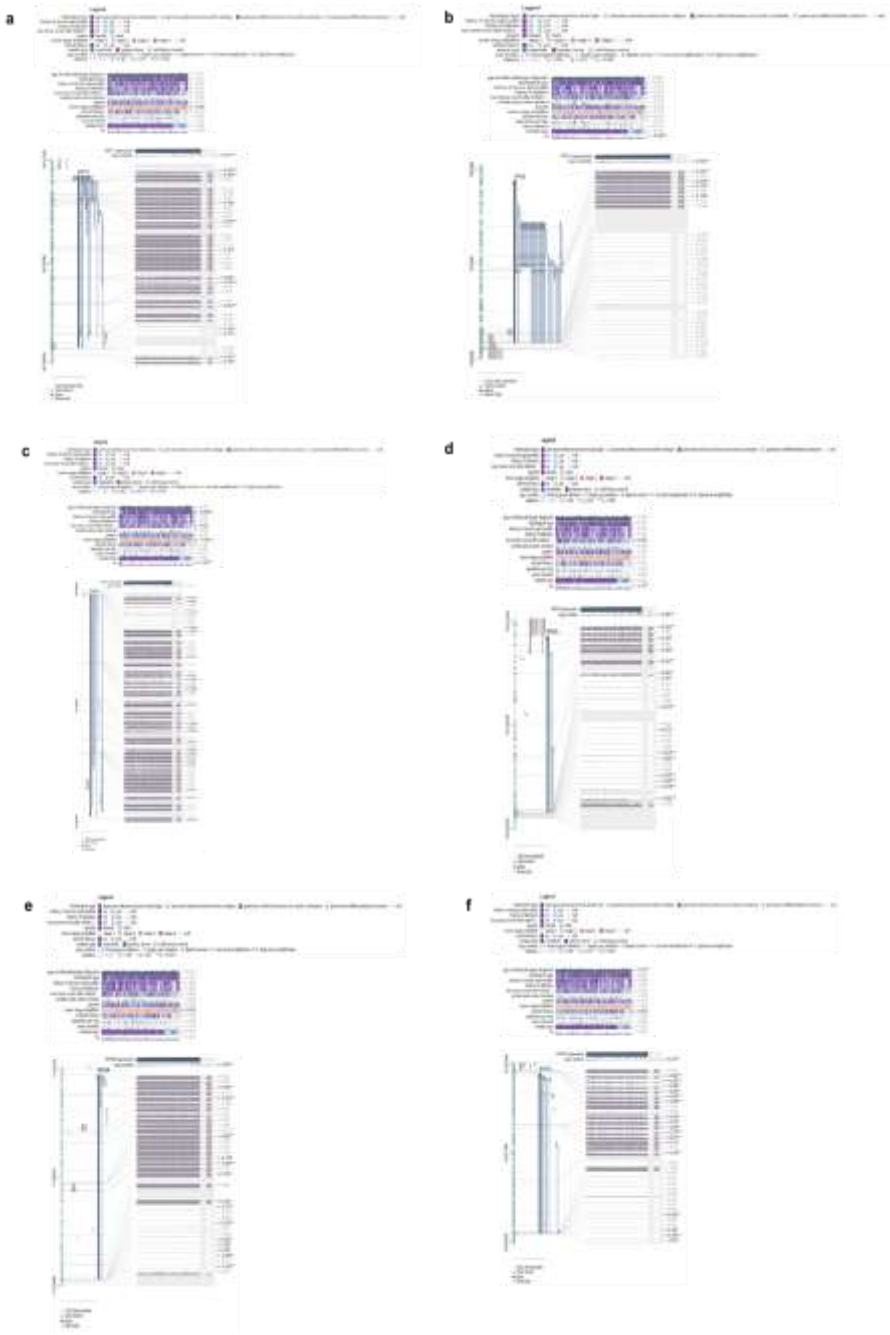

Figure 11. Methylation status exploration of hub genes via MEXPRESS in PAAD as well as normal samples. (a) AKT1 (b) TP53 (c)EGFR (d)KRAS (e)mTOR (f) STAT3.

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Druggability Assessment

Interaction between the gene and the drugs was done through the use of the DGIdb tool to determine the druggability potential of the hub genes identified. The analysis showed that all the six hub genes had interactions with numerous clinically approved as well as experimental small molecule drugs. Table 3 summarizes the clinically approved drugs associated with each gene. Among the hub genes, KRAS demonstrated the highest number of drug interactions (19 compounds), followed by EGFR (13 drugs), MTOR (6 drugs), STAT3 (6 drugs), and AKT1 and TP53 with one drug each.

These results underscore the therapeutic significance or status of identified hub genes and suggest that several, particularly KRAS and EGFR, could serve as promising candidates for targeted drug development and drug repurposing strategies in PAAD.

Table 3. Potential drug interactions of hub genes for future pharmacological targeting.

Gene	Clinically approved Drugs
AKT1	Capivasertib
TP53	Zanubrutinib
EGFR	Afatinib, Cetuximab, Necitumumab, Dacomitinib, Erlotinib, Icotinib, Neratinib, Lapatinib, Osimertinib, Panitumumab, Ramucirumab, Vandetanib, and Amivantamab.
KRAS	Panitumumab, Adagrasib, Danoprevir, Narlaprevir, Glecaprevir, Sotorasib, Simeprevir sodium, Vedroprevir, Grazoprevir, Paritaprevir, Tipiracil/Trifluridine, Teprotumumab, Asunaprevir, Faldaprevir, Voxilaprevir, Vaniprevir, Encorafenib, Telaprevir, Cetuximab.
mTOR	Everolimus, Temsirolimus, Zotarolimus, Sirolimus, Pimecrolimus, Ebastine.
STAT3	Bardoxolone methyl, Pyrimethamine, Osimertinib, Ouabain, Acitretin.

Regulatory Network Linking Hub Genes, Transcription Factors, and miRNAs

We analyzed the regulatory mechanisms of the recognized hub genes using Enrichr. Five transcription factors were identified to have regulatory influence over two or three of the hub genes, as summarized in Table 4. Furthermore, miRNA–gene interactions were explored using the miRTarBase database, revealing a total of 278 miRNAs related with the hub genes—62 targeting AKT1, 72 targeting TP53, 29 targeting EGFR, 49 targeting KRAS, 15 targeting MTOR, and 51 targeting STAT3. Finally, the transcription factors, miRNAs, as well as hub genes were integrated to construct a comprehensive gene–TF–miRNA regulatory network using Cytoscape, providing a holistic view of their regulatory interactions(Figure 12).

Table 4. Transcription factors regulating the identified hub genes

Transcription factor	Name	Genes	P-value
CRTC1	CREB-Regulated Transcription Coactivator 1	AKT1,TP53, EGFR	0.002821
RBPJ	Recombination Signal Binding Protein for Immunoglobulin Kappa J Region	AKT1, KRAS, TP53	0.007563
EGR1	Early Growth Response 1	TP53, EGFR	0.015853
CBFB	Core-Binding Factor Subunit Beta	STAT3, MTOR	0.020226
GATA1	GATA Binding Protein 1	STAT3, KRAS, TP53	0.035052

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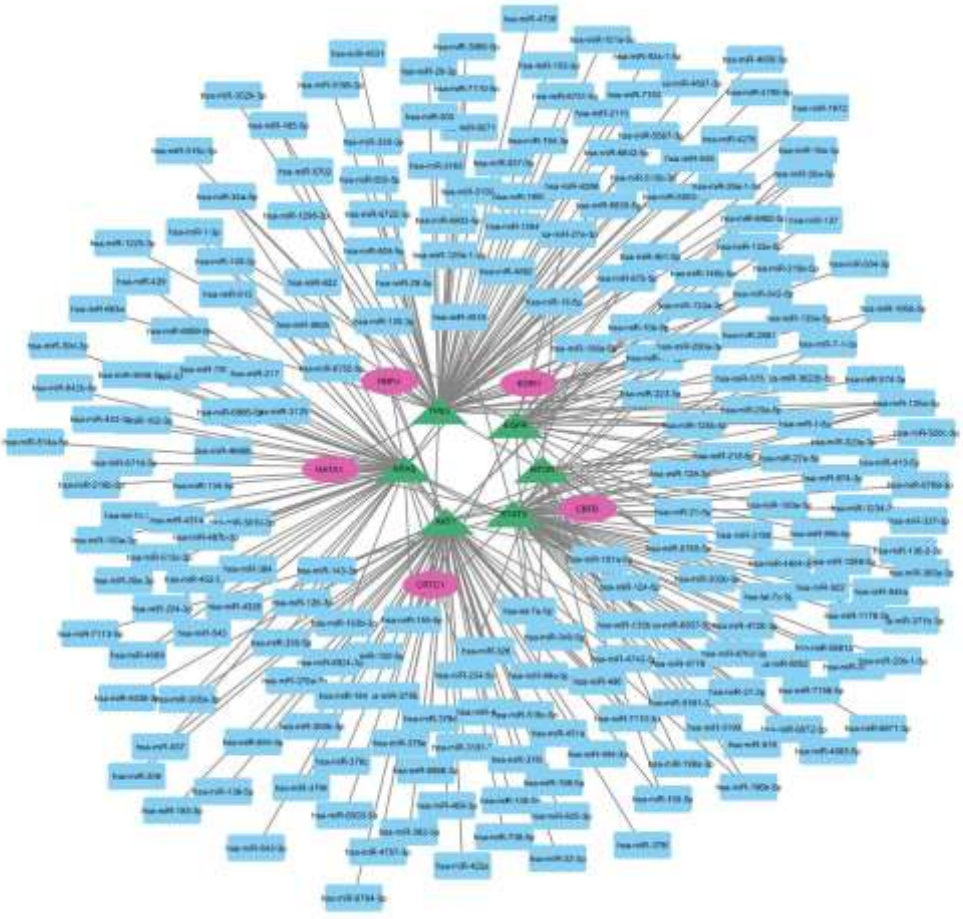

Figure 12. Overall network of the selected genes illustrating the regulatory relation with transcription factors and miRNAs. 6 genes (green triangles) were found associated with 5 transcription factors (pink elipse) and 278 miRNAs (blue rectangles) for their upstream regulation.

DISCUSSION

Pancreatic cancer is still among the top killers of cancer patients all over the globe because it is diagnosed at late level, has a very rapid progression, and is not sensitive to the conventional therapies(Zhao & Liu, 2020). To design strategies for diagnosis and treatment, it is crucial to understand the genetic, epigenetic and transcriptional changes that facilitate the progression of PDAC (Montalvo-Javé et al., 2023). Considering this, the identification of key hub genes for PAAD is helpful to the understanding of core molecular mechanisms involved in the development of tumors. We have identified 67 crucial pancreatic cancer-associated genes out of 19,275 genes obtained from two independent datasets. The network analysis of PPI showed that the network of pancreatic cancer–related genes were very dense, indicating the complexity of the PAAD signaling networks. Based on the results of our study, we identified AKT1, TP53, EGFR, KRAS, MTOR and STAT3 as the hub genes. For example, AKT1, EGFR, and MTOR control downstream pathways of PI3K–AKT–mTOR, a crucial pathway in the cancer cell growth and metabolism. On the other hand, it is known that mutations in KRAS are the primary occurrences that lead to the development of PDAC,while changes in TP53 and STAT3 genes are associated with tumor progression as well as immune evading mechanisms (Al-Hetty et al., 2023; Bansod et al., 2021; Stefanoudakis et al., 2024).

Both mRNA and protein expression analyses showed that hub genes in PAAD tissues were highly upregulated when compared to normal pancreatic tissues. thereby, indicating their key roles in tumor progression as well as initiation. The expression levels

of AKT1, KRAS, MTOR, and STAT3 increased with advancing cancer stages, whereas EGFR and KRAS were overexpressed in higher tumor grades, indicating their correlations with tumor aggressiveness and poor outcomes. Besides that, the terms of AKT1, KRAS, as well as STAT3 were meaningfully increased in the TP53-mutated samples, thereby suggesting their cooperative oncogenic effects. Protein expression analysis based on CPTAC data via UALCAN also showed that the levels of the six hub genes were elevated in tumor tissues, which is consistent with the mRNA expression profiles. These results were further confirmed with consistency across GEPIA, OncoDB and MuTarget databases. The activated transcription of hub genes is in line with previous studies that have shown that the activation of the PI3K-AKT-mTOR pathway, the KRAS pathway and the STAT3 pathway is the primary mechanisms of pancreatic cancer progression and resistance to treatment (Gurreri et al., 2023; Hamel et al., 2024; Stanciu et al., 2022). The correlation analysis has unveiled a robust co-expression pattern, which strongly implies that these genes are functionally interconnected and could be involved in the shared molecular signaling pathways in PAAD. The IHC results also support the transcriptional and proteomic upregulation of the hub genes identified in PAAD (Stanciu et al., 2022; Wee & Wang, 2017).

As a result of the immune infiltration analysis, it becomes evident that the selected hub genes are directly related to immune cell behavior in the PAAD microenvironment. The high positive correlations for AKT1, EGFR, KRAS, MTOR, and STAT3 genes with $CD8^+$ T cells, B cells, neutrophils, and macrophages indicate that these genes could be involved in immune cell recruitment and activation.

The relationship between hub genes' expression and immunosuppression markers, such as PD-L1 (CD274) and PD-1 (PDCD1), indicates that the selected hub genes play a role in immune escape in the PAAD microenvironment. Positive correlations, especially for EGFR, MTOR, KRAS, and STAT3 with the expression of PD-L1 are evidence of the fact that these hub genes could play a role in the immune checkpoint process, which leads to immunosuppression. It should be noted that EGFR pathways are extensively connected with PD-L1 upregulation, due to PI3K–AKT signaling activation (N. Zhang et al., 2016; W. Zhang et al., 2017).

The significant association of STAT3 with both PD-L1 and PD-1 further reinforces its role as a as a central immune-regulatory transcription factor that leads to tumor-induced immunosuppression (Bu et al., 2017). Conversely, the negative correlations observed for AKT1 and KRAS with PD-1 may indicate their indirect roles in immune modulation or context-dependent regulation of checkpoint molecules in PAAD. These results reveal that the hub genes, especially EGFR, MTOR, KRAS, and STAT3, are the main players in constructing the immunosuppressive milieu of pancreatic cancer (Guo & Wang, 2024). Their association with PD-L1 and PD-1 expression underscores their potential as therapeutic targets for combination approaches that participate molecular inhibition with immune checkpoint blockade to enhance immunotherapy efficacy in PAAD (Shi, 2018; Yin et al., 2023).

The survival analysis revealed distinct prognostic implications for the identified hub genes in PAAD. It is noteworthy that overexpression of EGFR, KRAS, MTOR, and STAT3 was significantly correlated with a higher HR and reduced overall survival, implying that these genes might be answerable for the tumorigenesis of the cancer cells, their ability to spread to distant sites, and resistance to cancer therapy (Du et al., 2025; Hu et al., 2024; Murugan, 2019; Shi et al., 2025).

It is known, from studies, that the EGFR and KRAS signaling pathways are activated in PAAD, and this results in the MAPK as well as PI3K–AKT–mTOR pathways being activated constitutively. These pathways are responsible for uncontrolled cell proliferation moreover apoptosis inhibition (de Jesus et al., 2023; Lee et al., 2016; Stanciu et al., 2022). Likewise, MTOR acts downstream of PI3K/AKT, enhancing cellular metabolism and survival under hypoxic conditions(Deng et al., 2023). At the same time, STAT3 is a transcriptional regulator of oncogenic and immunosuppressive genes that contributes to tumor progression and poor clinical outcomes (Rébé & Ghiringhelli, 2019).

The mutation profiling results emphasize the extensively known genetic landscape of PAAD, which mainly involves changes in KRAS and TP53. The extremely high mutation frequency of KRAS is consistent with its known role as a driver oncogene, thus being the primary cause of constitutive activation of the downriver signaling pathways for instance MAPK, PI3K–AKT, and STAT3 that lead to uncontrolled cell proliferation, metabolic reprogramming, and resistance to apoptosis (Huang et al., 2021; Ma et al., 2025; Parikh et al., 2022).

Mutations in TP53 similarly result in the loss of genomic stability, evasion of cell-cycle checkpoints, and impaired apoptotic regulation that further accelerate pancreatic tumor progression(Voutsadakis, 2021). In contrast, MTOR, STAT3, and AKT1 exhibited relatively low mutation frequencies.

The methylation analysis indicated that appearance of the hub genes in PAAD is influenced by both epigenetic and genetic mechanisms. Out of six hub genes, AKT1, KRAS, MTOR, and STAT3 had significant relationships between promoter methylation and gene expression, pointing to the fact that promoter methylation is one of the factors regulating their transcriptional activity. These findings imply that promoter methylation is a mechanism of gene regulation in some hub genes; however, genetic alterations such as amplification and mutation are still the major causes of their dysregulation in pancreatic cancer.

Druggability analysis has shown that the hub genes identified in the study carry high therapeutic potential and can interact with various clinically approved and other compounds. These results lead to the conclusion that the recognized hub genes might serve like potential therapeutic targets appropriate for the treatment of Pancreatic cancer.

Through the integrated analysis of mRNA and protein expression, correlation patterns, survival associations, immune cell infiltration, methylation, and mutation profiles, the hub genes that have been pinpointed turn out to be the key molecular players responsible for the progression of the Pancreatic cancer. Their combined regulatory influence reflects the complex interplay of genetic, epigenetic, and immunological mechanisms driving tumor development and maintenance. Together, these results serve the purpose of enlightening the pathogenesis of Pancreatic cancer which in turn is instrumental in the identification of diagnostic hub genes and the invention of targeted therapeutic strategies for the betterment of patient care.

CONCLUSION

An integrated bioinformatics approach was employed in this research to identify the key hub genes as well as the potential therapeutic targets linked with Pancreatic cancer. We discovered six hub genes that play critical roles in the molecular mechanisms underlying Pancreatic cancer. The combined results of functional enrichment, expression profiling, survival, immune infiltration, methylation, and druggability analyses demonstrated that these genes regulated tumor progression, immune regulation, and therapeutic response. This study suggests that the aforementioned hub genes can be used as a biomarker for diagnosis and treatment, as well as possible targets for therapy in Pancreatic cancer.

REFERENCES

[1] Al-Hetty, H. R. A. K., Abdulameer, S. J., Alkubaisy, S. A., Zaid, S. A., Jalil, A. T., & Jasim, I. K. (2023). STAT3 signaling in pancreatic ductal adenocarcinoma: a candidate therapeutic target. *Pathology, Research and Practice*, *245*(154425), 154425.

[2] Bansod, S., Dodhiawala, P. B., & Lim, K.-H. (2021). Oncogenic KRAS-induced feedback inflammatory signaling in pancreatic cancer: An overview and new therapeutic opportunities. *Cancers*, *13*(21), 5481.

[3] Bengtsson, A., Andersson, R., & Ansari, D. (2020). The actual 5-year survivors of pancreatic ductal adenocarcinoma based on real-world data. *Scientific Reports*, *10*(1), 16425.

[4] Bu, L. L., Yu, G. T., Wu, L., Mao, L., Deng, W. W., Liu, J. F., Kulkarni, A. B., Zhang, W. F., Zhang, L., & Sun, Z. J. (2017). STAT3 induces immunosuppression by upregulating PD-1/PD-L1 in HNSCC. *Journal of Dental Research*, *96*(9), 1027–1034.

[5] Cannon, M., Stevenson, J., Stahl, K., Basu, R., Coffman, A., Kiwala, S., McMichael, J. F., Kuzma, K., Morrissey, D., Cotto, K., Mardis, E. R., Griffith, O. L., Griffith, M., & Wagner, A. H. (2024). DGIdb 5.0: rebuilding the drug-gene interaction database for precision medicine and drug discovery platforms. *Nucleic Acids Research*, *52*(D1), D1227–D1235.

[6] Chandrashekar, D. S., Karthikeyan, S. K., Korla, P. K., Patel, H., Shovon, A. R., Athar, M., Netto, G. J., Qin, Z. S., Kumar, S., Manne, U., Creighton, C. J., & Varambally, S. (2022). UALCAN: An update to the integrated cancer data analysis platform. *Neoplasia (New York, N.Y.)*, *25*, 18–27.

[7] Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G. V., Clark, N. R., & Ma'ayan, A. (2013). Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*, *14*(1), 128.

[8] Cho, M., Tang, G., Rogers, C. S., Dove, M., Liu, X., Li, Y., & Wang, X. (2025). OncoDB 2.0: a comprehensive platform for integrated pan-cancer omics analysis. *Nucleic Acids Research*, *gkaf952*. https://doi.org/10.1093/nar/gkaf952

[9] Davis, A. P., Wiegers, T. C., Sciaky, D., Barkalow, F., Strong, M., Wyatt, B., Wiegers, J., McMorran, R., Abrar, S., & Mattingly, C. J. (2025). Comparative Toxicogenomics Database's 20th anniversary: update 2025. *Nucleic Acids Research*,

53(D1), D1328–D1334.

[10] de Bruijn, I., Kundra, R., Mastrogiacomo, B., Tran, T. N., Sikina, L., Mazor, T., Li, X., Ochoa, A., Zhao, G., Lai, B., Abeshouse, A., Baiceanu, D., Ciftci, E., Dogrusoz, U., Dufilie, A., Erkoc, Z., Garcia Lara, E., Fu, Z., Gross, B., … Schultz, N. (2023). Analysis and visualization of longitudinal genomic and clinical data from the AACR Project GENIE Biopharma Collaborative in cBioPortal. *Cancer Research*, *83*(23), 3861–3867.

[11] de Jesus, V. H. F., Mathias-Machado, M. C., de Farias, J. P. F., Aruquipa, M. P. S., Jácome, A. A., & Peixoto, R. D. (2023). Targeting KRAS in pancreatic ductal adenocarcinoma: The long road to cure. *Cancers*, *15*(20), 5015.

[12] Deng, H., Chen, Y., Li, P., Hang, Q., Zhang, P., Jin, Y., & Chen, M. (2023). PI3K/AKT/mTOR pathway, hypoxia, and glucose metabolism: Potential targets to overcome radioresistance in small cell lung cancer. *Cancer Pathogenesis and Therapy*, *1*(1), 56–66.

[13] Digre, A., & Lindskog, C. (2021). The Human Protein Atlas-Spatial localization of the human proteome in health and disease. *Protein Science: A Publication of the Protein Society*, *30*(1), 218–233.

[14] Dong, H., Zhang, S., Wei, Y., Liu, C., Wang, N., Zhang, P., Zhu, J., & Huang, J. (2018). Bioinformatic analysis of differential expression and core GENEs in breast cancer. *International Journal of Clinical and Experimental Pathology*, *11*(3), 1146–1156.

[15] Du, Y., Karatekin, F., Wang, W. K., Hong, W., & Boopathy, G. T. K. (2025). Cracking the EGFR code: Cancer biology, resistance mechanisms, and future therapeutic frontiers. *Pharmacological Reviews*, *77*(5), 100076.

[16] Ge, S. X., Jung, D., & Yao, R. (2020). ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics (Oxford, England)*, *36*(8), 2628–2629.

[17] Glaviano, A., Foo, A. S. C., Lam, H. Y., Yap, K. C. H., Jacot, W., Jones, R. H., Eng, H., Nair, M. G., Makvandi, P., Geoerger, B., Kulke, M. H., Baird, R. D., Prabhu, J. S., Carbone, D., Pecoraro, C., Teh, D. B. L., Sethi, G., Cavalieri, V., Lin, K. H., … Kumar, A. P. (2023). PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Molecular Cancer*, *22*(1), 138.

[18] Guo, S., & Wang, Z. (2024). Unveiling the immunosuppressive landscape of pancreatic ductal adenocarcinoma: implications for innovative immunotherapy strategies. *Frontiers in Oncology*, *14*, 1349308.

[19] Gurreri, E., Genovese, G., Perelli, L., Agostini, A., Piro, G., Carbone, C., & Tortora, G. (2023). KRAS-dependency in pancreatic ductal adenocarcinoma: Mechanisms of escaping in resistance to KRAS inhibitors and perspectives of therapy. *International Journal of Molecular Sciences*, *24*(11), 9313.

[20] Hamel, Z., Sanchez, S., Standing, D., & Anant, S. (2024). Role of STAT3 in pancreatic cancer. *Exploration of Targeted Anti-Tumor Therapy*, *5*(5), 20–34.

[21] Heberle, H., Meirelles, G. V., da Silva, F. R., Telles, G. P., & Minghim, R. (2015). InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics*, *16*(1), 169.

[22] Huang, L., Guo, Z., Wang, F., & Fu, L. (2021). KRAS mutation: from undruggable to druggable in cancer. *Signal Transduction and Targeted Therapy*, *6*(1), 386.

[23] Hu, Y., Dong, Z., & Liu, K. (2024). Unraveling the complexity of STAT3 in cancer: molecular understanding and drug discovery. *Journal of Experimental & Clinical Cancer Research: CR*, *43*(1), 23.

[24] Jafari, S., Ravan, M., Karimi-Sani, I., Aria, H., Atapour, A., & Sarab, G. A. (2022). Screening and identification of potential biomarkers for pancreatic cancer: An integrated bioinformatics analysis. In *Research Square*. https://doi.org/10.21203/rs.3.rs-1841248/v1

[25] Karimi, E., Kalaki, N. S., & Akrami, S. M. (2025). Identifying Hub Genes and Pathways in Pancreatic Ductal Adenocarcinoma (PAAD): A comprehensive in silico study. *Biochemistry and Biophysics Reports*, *41*(101921), 101921.

[26] Kaur, S., Baine, M. J., Jain, M., Sasson, A. R., & Batra, S. K. (2012). Early diagnosis of pancreatic cancer: challenges and new developments. *Biomarkers in Medicine*, *6*(5), 597–612.

[27] Koch, A., De Meyer, T., Jeschke, J., & Van Criekinge, W. (2015). MEXPRESS: visualizing expression, DNA methylation and clinical TCGA data. *BMC Genomics*, *16*(1), 636.

[28] Lee, S., Heinrich, E. L., Lu, J., Lee, W., Choi, A. H., Luu, C., Chung, V., Fakih, M., & Kim, J. (2016). Epidermal growth factor receptor signaling to the mitogen activated protein kinase pathway bypasses Ras in pancreatic cancer cells. *Pancreas*, *45*(2), 286–292.

[29] Li, T., Fan, J., Wang, B., Traugh, N., Chen, Q., Liu, J. S., Li, B., & Liu, X. S. (2017). TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Research*, *77*(21), e108–e110.

[30] Ma, Q., Zhang, W., Wu, K., & Shi, L. (2025). The roles of KRAS in cancer metabolism, tumor microenvironment and clinical therapy. *Molecular Cancer*, *24*(1), 14.
[31] Montalvo-Javé, E. E., Nuño-Lámbarri, N., López-Sánchez, G. N., Ayala-Moreno, E. A., Gutierrez-Reyes, G., Beane, J., & Pawlik, T. M. (2023). Pancreatic cancer: Genetic conditions and epigenetic alterations. *Journal of Gastrointestinal Surgery: Official Journal of the Society for Surgery of the Alimentary Tract*, *27*(5), 1001–1010.
[32] Murugan, A. K. (2019). mTOR: Role in cancer, metastasis and drug resistance. *Seminars in Cancer Biology*, *59*, 92–111.
[33] Nagy, Á., & Győrffy, B. (2021). muTarget: A platform linking gene expression changes and mutation status in solid tumors. *International Journal of Cancer. Journal International Du Cancer*, *148*(2), 502–511.
[34] Parikh, K., Banna, G., Liu, S. V., Friedlaender, A., Desai, A., Subbiah, V., & Addeo, A. (2022). Drugging KRAS: current perspectives and state-of-art review. *Journal of Hematology & Oncology*, *15*(1), 152.
[35] Posta, M., & Győrffy, B. (2025). Pathway-level mutational signatures predict breast cancer outcomes and reveal therapeutic targets. *British Journal of Pharmacology*, *bph.70215*. https://doi.org/10.1111/bph.70215
[36] Qin, G., & Zhao, X.-M. (2014). A survey on computational approaches to identifying disease biomarkers based on molecular networks. *Journal of Theoretical Biology*, *362*, 9–16.
[37] Rébé, C., & Ghiringhelli, F. (2019). STAT3, a master regulator of anti-tumor immune response. *Cancers*, *11*(9), 1280.
[38] Ru, B., Wong, C. N., Tong, Y., Zhong, J. Y., Zhong, S. S. W., Wu, W. C., Chu, K. C., Wong, C. Y., Lau, C. Y., Chen, I., Chan, N. W., & Zhang, J. (2019). TISIDB: an integrated repository portal for tumor-immune system interactions. *Bioinformatics (Oxford, England)*, *35*(20), 4200–4202.
[39] Sarantis, P., Koustas, E., Papadimitropoulou, A., Papavassiliou, A. G., & Karamouzis, M. V. (2020). Pancreatic ductal adenocarcinoma: Treatment hurdles, tumor microenvironment and immunotherapy. *World Journal of Gastrointestinal Oncology*, *12*(2), 173–181.
[40] Shi, Y. (2018). Regulatory mechanisms of PD-L1 expression in cancer cells. *Cancer Immunology, Immunotherapy: CII*, *67*(10), 1481–1489.
[41] Shi, Y., Zheng, H., Wang, T., Zhou, S., Zhao, S., Li, M., & Cao, B. (2025). Targeting KRAS: from metabolic regulation to cancer treatment. *Molecular Cancer*, *24*(1), 9.
[42] Stanciu, S., Ionita-Radu, F., Stefani, C., Miricescu, D., Stanescu-Spinu, I.-I., Greabu, M., Ripszky Totan, A., & Jinga, M. (2022). Targeting PI3K/AKT/mTOR signaling pathway in pancreatic cancer: From molecular to clinical aspects. *International Journal of Molecular Sciences*, *23*(17), 10132.
[43] Stefanoudakis, D., Frountzas, M., Schizas, D., Michalopoulos, N. V., Drakaki, A., & Toutouzas, K. G. (2024). Significance of TP53, CDKN2A, SMAD4 and KRAS in pancreatic cancer. *Current Issues in Molecular Biology*, *46*(4), 2827–2844.
[44] Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., Fishilevich, S., Stein, T. I., Nudel, R., Lieder, I., Mazor, Y., Kaplan, S., Dahary, D., Warshawsky, D., Guan-Golan, Y., Kohn, A., Rappaport, N., Safran, M., & Lancet, D. (2016). The GeneCards suite: From gene data mining to disease genome sequence analyses. *Et Al [Current Protocols in Bioinformatics]*, *54*(1), 1.30.1–1.30.33.
[45] Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & von Mering, C. (2023). The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, *51*(D1), D638–D646.
[46] Tang, Z., Li, C., Kang, B., Gao, G., Li, C., & Zhang, Z. (2017). GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Research*, *45*(W1), W98–W102.
[47] Vandenbrouck, Y., Christiany, D., Combes, F., Loux, V., & Brun, V. (2019). Bioinformatics tools and workflow to select blood biomarkers for early cancer diagnosis: An application to pancreatic cancer. *Proteomics*, *19*(21-22), e1800489.
[48] Vareedayah, A. A., Alkaade, S., & Taylor, J. R. (2018). Pancreatic adenocarcinoma. *Missouri Medicine*, *115*(3), 230–235.
[49] Voutsadakis, I. A. (2021). Mutations of p53 associated with pancreatic cancer and therapeutic implications. *Annals of Hepato-Biliary-Pancreatic Surgery*, *25*(3), 315–327.
[50] Wee, P., & Wang, Z. (2017). Epidermal growth factor receptor cell proliferation signaling pathways. *Cancers*, *9*(5), 52.
[51] Yin, S., Chen, Z., Chen, D., & Yan, D. (2023). Strategies targeting PD-L1 expression and associated opportunities for cancer combination therapy. *Theranostics*, *13*(5), 1520–1544.
[52] Zhang, N., Zeng, Y., Du, W., Zhu, J., Shen, D., Liu, Z., & Huang, J.-A. (2016). The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung

cancer. *International Journal of Oncology*, *49*(4), 1360–1368.

[53] Zhang, W., Pang, Q., Yan, C., Wang, Q., Yang, J., Yu, S., Liu, X., Yuan, Z., Wang, P., & Xiao, Z. (2017). Induction of PD-L1 expression by epidermal growth factor receptor-mediated signaling in esophageal squamous cell carcinoma. *OncoTargets and Therapy*, *10*, 763–771.

[54] Zhao, Z., & Liu, W. (2020). Pancreatic cancer: A review of risk factors, diagnosis, and treatment. *Technology in Cancer Research & Treatment*, *19*, 1533033820962117.