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The DEAD-box RNA helicase, DDX60, Suppresses immunotherapy and promotes malignant progression of pancreatic cancer

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ABSTRACT

Excessive proliferation, invasion, metastasis, and immune resistance in pancreatic cancer (PC) makes it one of the most lethal malignant tumors. Recently, DDX60 was found to be involved in the development of various tumors and in immunotherapy. Therefore, we aimed to investigate whether DDX60 is a new factor involved in PC immunotherapy. The *DDX60* mRNA was screened using transcriptome sequencing (RNA-seq). The Cox and survival analysis of DDX60 was performed using the Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases. In addition, clinical and immune infiltration data in the databases were analyzed and plotted using the R language. Clinical samples and in vitro experiments were used to determine the molecular evolution of DDX60 during PC progression. We found that DDX60 was upregulated in PC tissues (P value = 0.0083) and was associated with poor prognosis and short survival time of patients with PC. Results of Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and gene set variation analyses showed that viral defense, tumor, and immune-related pathways were significantly enriched in samples with high DDX60 expression. The Pearson correlation test demonstrated that DDX60 expression correlated strongly with immune checkpoint and immune system-related metagene clusters. Our results indicated that DDX60 promoted cell proliferation, migration, and invasion and was related to poor prognosis and immune resistance. Therefore, DDX60 may be a promising novel target for PC immunotherapy.

Pancreatic cancer (PC) remains a highly malignant tumor, with a 5-year survival rate as low as 9% [1]. For patients with resectable disease at presentation, surgery is the standard treatment followed [2]. However, a vast majority of patients are diagnosed with advanced unresectable disease, early tumor invasion, and resistance to chemotherapy, and most patients experience recurrence soon after treatment, resulting in poor treatment outcome [3]. One American study projected that PC would become the second-leading cause of cancer-related mortality by 2030 [4]. Therefore, comprehensive diagnosis and adjuvant therapies should be optimized to enable early detection of cases and avoid poor outcomes.

Tumor immunotherapy has brought new hope to patients and achieved breakthroughs in both research and clinical practice. Immune checkpoint inhibitors that recognize cell surface receptors and ligands prevent immunosuppressive signaling pathways and enhance the immune response against tumors [5]. Adoptive cellular therapy promotes the aggregation of and cytokine production by T-cells; in this approach, patient cell-derived therapeutic cancer vaccines are used to eliminate

Abbreviations: PC, pancreatic cancer; TCGA, The Cancer Genome Atlas; GSVA, Gene set variation analysis; GEO, Gene Expression Omnibus; GEPIA, Gene Expression Profiling Interactive Analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DDX, RNA helicase DEAD box 1 Introduction.

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tumors [6,7]. Indeed, the potential of cancer immunotherapies is vast and they have been successfully used in treating multiple solid and hematological malignancies, such as melanoma, renal cell carcinoma, and refractory B-cell precursor acute lymphoblastic leukemia [8]. The effect of immunotherapy on PC is not stable, which may be related to the tolerance of PC to immunotherapy, the molecular mechanism of which remains unclear [9].

The RNA helicase DEAD box (DDX) family belongs to superfamily 2, the largest family of RNA lyases. The DDX family is characterized by a consensus Asp-Glu-Ala-Asp (DEAD) motif, and the human genome encodes 37 DDX members [10]. Previous studies have demonstrated that DDX plays an essential role in all cellular processes, such as cell cycle progression, apoptosis, innate immune response, viral replication, and tumorigenesis [11,12]. In cancer cells, DDXs increase the expression of oncogenes and decrease the production of tumor suppressors, thereby promoting progression of cancer [13]. DDX60, a member of the DEAD-box family, is a transcription factor that plays an important role in human antiviral activity and interferon immunity [14]. In addition, studies have shown that DDX60 participates in intestinal immune response, colorectal cancer immunotherapy, and breast cancer radiosensitivity [15–17]. It participates in the chemoprevention of PC via the cholecystokinin-2 receptor as an intermediate [18]. DDX60 may act as a potential immune therapeutic target in glioma.

However, there is a lack of reports regarding the role of DDX60 in patients with PC. The aim of this study was to reveal the mechanism of DDX60 in pancreatic cancer by using bioinformatics databases and cell experiments. We believe that this study is crucial to determine the potential biomarkers for immunotherapy of pancreatic cancer in future medical research.

1. Materials and methods

1.1. Patients and samples

The collection of samples and clinical information in this study were approved by the Affiliated Hospital of Jiangnan University, and written informed consent was obtained from each patient. Two cohorts were used: the PC cohort 1 contained three pairs of pancreatic samples and adjacent non-tumor samples, and the PC cohort 2 contained 37 pairs of samples.

1.2. Bioinformatics analysis

The gene expression profile of the DDX family was analyzed using the online tool, Gene Expression Profile Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) [19], and downloaded data from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/)-GSE62452 dataset [20] containing 69 pancreatic tumors and 61 adjacent non-tumor tissues. The gene expression data for PC were also downloaded from The Cancer Genome Atlas (TCGA) online database (http://tcga-data.nci.nih.gov/tcga/) using data from 178 cases. Pan-cancer analysis and histochemistry images of DDX60 were obtained from the TNMplot and Human Protein Atlas database [21,22]. All TCGA and GEO data were calculated and processed using R (http://www.r-project.org) and the IBM SPSS (Statistical Program for Social Sciences) Statistics (version 25.0) software [23].

1.3. Functional enrichment analysis

A list of the most relevant or characteristic genes of the cell clusters of DDX60 was uploaded to the Database for Annotation, Visualization, and Integrated Discovery (DAVID 2021) [24]. The official gene symbol was selected as the identifier, and *Homo sapiens* was selected as the species. Finally, the enrichment results of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were obtained. This study shows the top six results in ascending order of P values ($P < 0.05$), and the pictures were generated using the ggplot 2 package and EXCEL office 2019.

1.4. Gene set variation analysis (GSVA)

The list of immune process-related genes was obtained from the Gene Set Enrichment Analysis (GSEA version 4.3.2) website (http://www.gsea-msigdb.org/) [25,26]. The functional enrichment score was calculated for each PC sample using the package of R environment. Thermal mapping of enrichment results was performed using the pheatmap package (R environment). Pearson's correlation analysis was used to determine the correlation between DDX60 levels and immune processes.

1.5. Transcriptome sequencing (RNA-seq)

The surgical specimens of the three patients with cancer were rapidly soaked in liquid nitrogen for preservation. Sample labeling and array hybridization were performed according to the Agilent One-Color microarray-based gene expression analysis protocol (Agilent Technology), with minor modifications. Briefly, mRNA was purified from total RNA after removal of rRNA (mRNA-ONLY™ eukaryotic mRNA isolation kit, Epicenter). The hybridized arrays were washed, fixed, and scanned using an Agilent DNA microarray scanner (part number G2505C). RNA quantity and quality were measured using a NanoDrop ND-1000. RNA integrity was assessed using standard denaturing agarose gel electrophoresis.

1.6. Cell lines and cultures

The PC cell line, BxPC-3, was purchased from Procell Life Science & Technology Co. Ltd. (Wuhan, China) and cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, USA) containing 10% fetal calf serum (Gemini, USA), 100 μg/mL streptomycin (Sangon Co. Ltd., Shanghai, China), and 100 U/mL penicillin (Sangon Co. Ltd., Shanghai, China) at 37 °C in a 5% CO_2 incubator.

1.7. Transfection

The DDX60 siRNA was purchased from PROTEINBIO (Nanjing, China). The siRNAs were transfected using RFect small nucleic acid transfection reagent (BAIDAI, Changzhou, China), according to the manufacturer's instructions. DDX60 expression was confirmed using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blotting. The sequences of the siRNAs used for knocking down *DDX60* are listed in Supplementary Table S1.

1.8. RT-qPCR

The total RNA from cultured cells with or without *DDX60* knockdown or 37 pairs of tissue specimens was isolated using the FastPure® cell/tissue total RNA isolation kit V2 (Vazyme, Nanjing, China). RNA was then reverse transcribed into cDNA using the HIScript®III RT SuperMix for Qpcr (+gDNA wiper) (Vazyme, Nanjing, China). ChamQ Universal SYBR qPCR master mix (Vazyme) was used for PCR using an ABI 7500 PC R system (ABI Co. Ltd., USA). The relative RNA levels of candidate genes were calculated using the $2^{-\Delta\Delta Ct}$ method. The sequences of the primers used are listed in Supplementary Table S2. Each sample was examined in triplicates. The specificity of the PCR product was confirmed using melting curve analyses.

1.9. Western blot analysis

Protein expression was analyzed using western blotting. Briefly, total protein was extracted using an immunoprecipitation protein lysis buffer (Beyotime Biotechnology, Shanghai, China). Protein concentration was determined using the bicinchoninic acid protein determination kit

(Vazyme, Nanjing, China). Protein samples (100 μg) were loaded onto 10% sodium dodecyl sulfate-polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Amersham Biosciences, Buckinghamshire, UK) for 150 min. The primary antibodies were incubated in blocking buffer overnight at 4 °C. The primary antibodies used were anti-β-actin (1:10,000 dilution; Abcam, Shanghai, China) and anti-DDX60 (1:10,000 dilution; GeneTex, USA). The Image J software (National Institutes of Health, USA) was used for densitometric analyses. The assay was performed in triplicate.

1.10. Cell counting kit- 8 (CCK-8) assay

The CCK-8 kit (Vazyme, Nanjing, China) was used to assay cell viability. The transfected cells (1×10^4 cells per well) were incubated in 96-well plates in 100 μL complete growth medium for 1–5 days. Then, 10 μL CCK-8 reagent was added to each well every day and incubated for 2 h. The optical density at 450 nm was measured using a Multiskan reader (Thermo Scientific, USA). Three replicates were performed for each CCK-8 assay.

1.11. Transwell assays

Cell migration was examined using the transwell assay. 150 μL, 5×10^4 cells in serum-free DMEM medium (HyClone, USA) were added to the upper chamber containing an 8 μm polycarbonate filter (LABSELECT, Beijing, China). The lower chamber was filled with DMEM supplemented with 10% fetal bovine serum. After incubation at 37 °C in the presence of 5% CO_2 for 24 h, the non-migrated cells at the top surface were removed. The invading cells on the lower surface were fixed with 4% paraformaldehyde for 15 min and stained with 0.1% crystal violet for 20 min. Images were captured and cells were counted under an inverted microscope (magnification, × 20; Olympus Corporation, Japan). The cell invasion capacity was examined using transwell chambers coated with 80 μL BD Biosciences Matrigel (1:8 dilution) for 4 h in a 5% CO_2 incubator.

1.12. Statistical analysis

All statistical analyses and visualizations were performed using R (version 4.2.2), IBM SPSS Statistics (version 25.0) software, and GraphPad Prism 9.0.2. An unpaired *t*-test was used to compare the significance of differences between the two groups. The significance of the differences between more than two groups was determined using one-way analysis of variance (ANOVA). Correlation analysis between the two groups was performed using Pearson correlation analysis. Survival curves were constructed using the Kaplan-Meier method (log-rank test). The prognostic independent risk factors were evaluated using the Cox regression method (proportional hazards model). Statistical significance

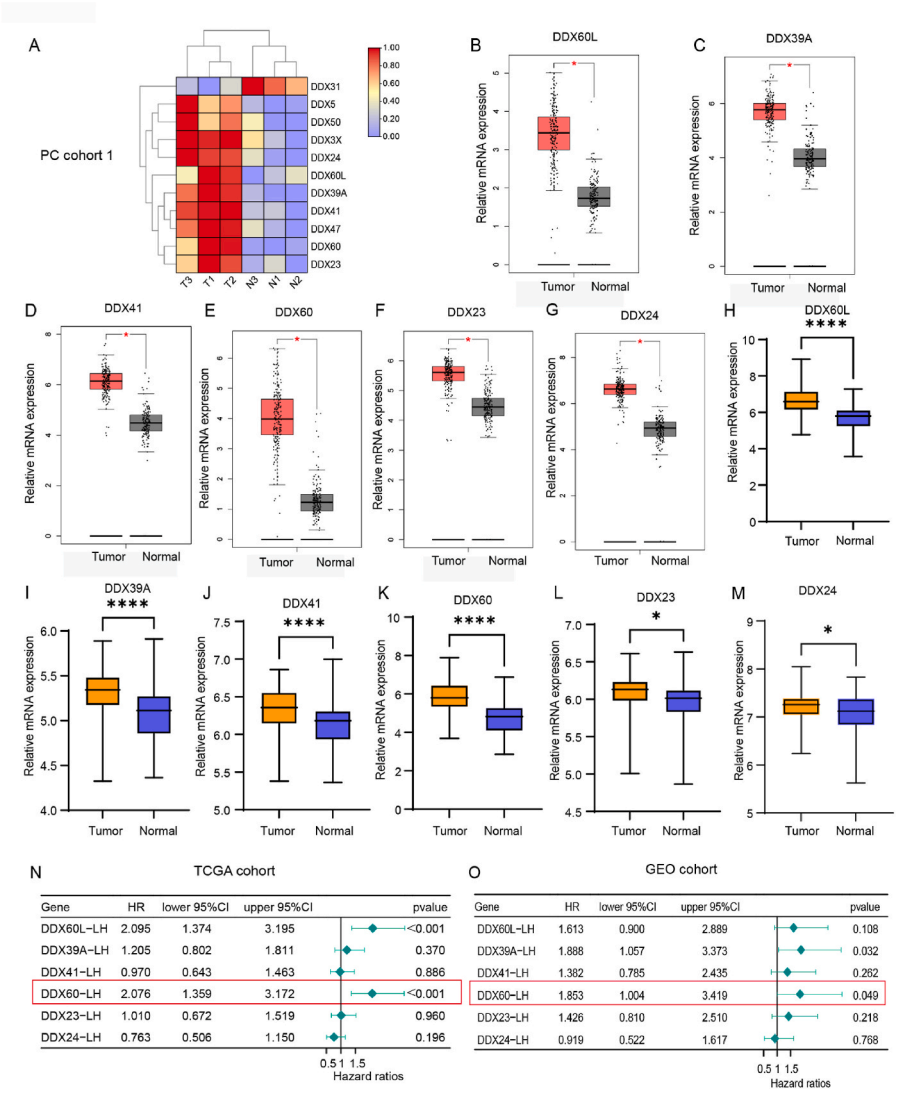

Fig. 1. Expression of DDX family in tumor tissues and adjacent normal pancreatic tissues. (A) RNA microarray identified differentially expressed DDX family members in PC tissues (T1, T2, T3) compared to that in adjacent normal pancreatic tissues (N1, N2, N3). **(B–G)** Expression of *DDX60L, DDX39A, DDX41, DDX60, DDX23* and *DDX24* in the GEPIA database. **(H–M)** Expression of *DDX60L, DDX39A, DDX41, DDX60, DDX23* and *DDX24* in the GSE62452 dataset. *P < 0.05, **P < 0.01, ***P < 0.001. ****P < 0.0001. **(N)** Univariate analysis of *DDX60L, DDX39A, DDX41, DDX60, DDX23* and *DDX24* in patients with PC from The Cancer Genome Atlas cohort using a Cox regression model. **(O)** Univariate analysis of *DDX60L, DDX39A, DDX41, DDX60, DDX23* and *DDX24* in patients with PC from the Gene Expression Omnibus cohort using a Cox regression model. LH: Lower and Higher; the cutoff of the group is the median expression of genes.

was set at $P < 0.05$.

2. Results

2.1. Expression of DDX family in tumor tissues and adjacent normal pancreatic tissues

To investigate the functional implications of the DDX family in PC, we performed transcriptome sequencing to compare the gene expression profiles in PC tissues and adjacent normal pancreatic tissues (PC cohort 1). The results revealed that 10 genes of DDX family were expressed at significantly higher levels in PC tissues than in normal tissue (Fig. 1A). We queried the published clinical databases, GEPIA and GEO dataset GSE62452, to further analyze the expression of these genes and found that in both databases, *DDX60 L/39 A/41/60/23/*24 mRNA expression was significantly higher in PC tissues than in normal tissues (Fig. 1B-M). However, the levels of other DDX family proteins in the two datasets were inconsistent or did not differ significantly difference (Supplementary Fig. S1). Furthermore, Cox regression analysis of TCGA cohort and GSE62452 survival data suggested that only DDX60 showed a significant difference in predicting survival time (Fig. 1N and O). Therefore, we chose to perform the remaining analysis on DDX60.

2.2. DDX60 is overexpressed in pancreatic cancer and related to short overall survival (OS)

To analyze DDX60 expression, we queried the TNMplot database and found that DDX60 mRNA was overexpressed in multiple cancers (Fig. 2A) and that DDX60 level was significantly higher in PC tumor and metastatic tissues than in normal tissues (Fig. 2B). Using The Human Protein Atlas database, the above results for PC were further investigated at the protein level (Fig. 2C). To confirm these findings, we validated the bioinformatics data in a sample cohort consisting of 37 pairs of PC tissues (PC cohort 2) using RT-qPCR. The mRNA level of *DDX60* was significantly higher in PC tissues (Fig. 2D). Kaplan-Meier and Cox proportional hazard model analyses were performed to investigate the predictive value of DDX60 in patients with PC. Based on the median DDX60 expression as the cut-off value, patients with higher expression of DDX60 were found to have significantly shorter OS than those with lower DDX60 expression (Fig. 2E and F). Both univariate and multivariate analyses of TCGA cohort revealed that DDX60 is an independent prognostic factor for reduced OS (Supplementary Table S3). These results suggested that DDX60 expression is an independent prognostic factor that can effectively predict the prognosis of patients with PC.

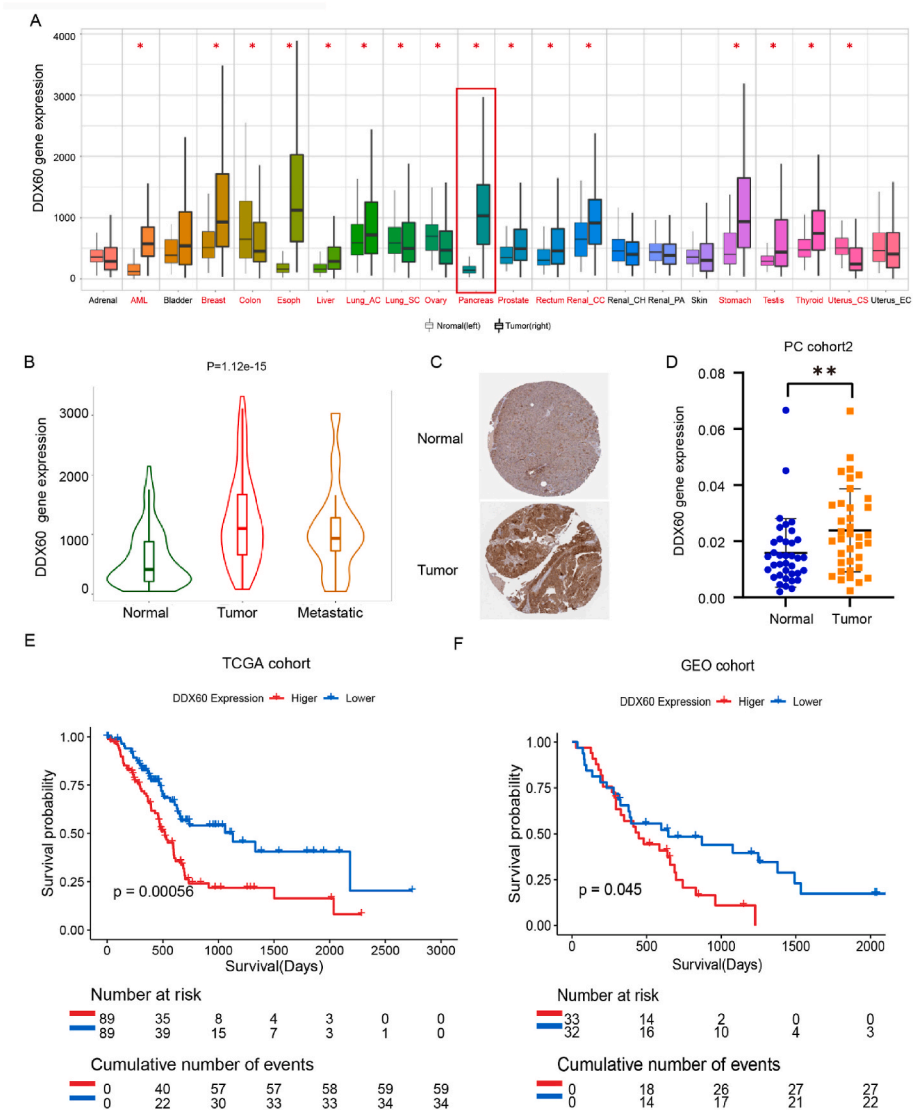

Fig. 2. DDX60 is overexpressed in pancreatic cancer and related to short overall survival (OS). **(A)** DDX60 expression levels in different tumor types from the TNMplot database. *P < 0.05, **P < 0.01, ***P < 0.001. **(B)** DDX60 mRNA levels in PC tumor, metastatic, and normal tissues in the TNMplot database. **(C)** Representative images of immunohistochemical staining for DDX60 in tissue microarrays constructed from PC samples. Scale bar, 200 μm. **(D)** mRNA levels of DDX60 in 37 PC tissues and paired normal tissues (PC cohort 2) were determined using RT-qPCR. P = 0.0083. **(E, F)** Kaplan-Meier analysis of DDX60 expression. The cut-off for the group was the median expression of DDX60. The significance of prognostic value was tested using the log-rank test.

2.3. Functional analysis of DDX60 in patients with pancreatic cancer

To further investigate the molecular characteristics of DDX60, the genes most related to DDX60 were screened using Pearson correlation analysis ($|R| > 0$ 0.5, $P < 0.05$) in the TCGA and GEO cohorts. GO and KEGG analyses were performed based on the gene sets described above. In TCGA cohort, biological processes most related to DDX60 included defense response to virus, innate immune response and type I interferon signaling pathway (Fig. 3A). The cytoplasm was the most related cellular component (Fig. 3B). And the molecular functions were found to be RNA binding and 2′-5′-oligoadenylate synthetase activity (Fig. 3C). Response to viral signaling pathways were the pathways most related to DDX60 (Fig. 3D). DDX60 in the GEO cohort were similar to those in TCGA cohort (Fig. 3E-H). Previous studies have suggested that DDX family is involved in immune responses to tumors [27]. Therefore, we tested the effects of DDX60 activation on the immune pathways and cytokine gene sets. Based on the enrichment results, we selected multiple gene sets related to immune processes and cytokine regulation, and GSVA analysis was used to determine the enrichment score of the immune process, the correlation analysis showed that DDX60 expression correlated positively with most immune functions in TCGA database (Fig. 3I and J). Based on these results, we hypothesized that DDX60 was involved in immune response in PC and may possibly affect the efficacy of immunotherapy.

2.4. DDX60 expression was related to the immune infiltration of pancreatic cancer

Immune cell infiltration in the tumor microenvironment plays a key role in tumor development, and immune cytokines mediate key interactions between immune and non-immune cells, which affects the clinical outcome [28,29]. To further study the regulatory function of DDX60 expression on the immune response of cancer cells, we used the R software to analyze the infiltration of immune cells in TCGA and GEO cohorts (Fig. 4A and B). We then analyzed the relationship between expression of the DDX family proteins and tumor immune cells in PC (Fig. 4C and D), which suggested that DDX60 correlated negatively with many immune cells, including $CD8^+$ T cells and natural killer cells. In contrast, DDX60 was positively activated by M2 macrophages and activated dendritic cells. Then, the relationship between DDX60 and primary inhibitory immune checkpoints (TIM-3, PD-1, HVEM, CD200R1, PD-2, CTLA-4, TIGIT, and CD47) were studied in the TCGA and GEO cohorts (Fig. 4E). DDX60 was strongly positively correlated with most inhibitory immune checkpoints, which may indicate the suppression of the immune process against PC. Besides, we selected eight metagene clusters associated with the immune system as markers of immune status. These include hematopoietic cell kinase (HCK), IgG, interferon, lymphocyte-specific kinase (LCK), major histocompatibility complex I (MHC-I), major histocompatibility complex II (MHC- II), signal transducer and activator of transcription 1 (STAT1) and signal transducer and activator of transcription 2 (STAT2) (Supplementary file 1) [23,30]. Related diagrams showed that DDX60 correlated significantly and positively with most clusters, with the exception of IgG, in TCGA and GEO databases (Fig. 4F and G). Considering that IgG is primarily associated with B lymphocyte activity, the expression of DDX60 as an immunosuppressive factor increased when macrophages and T cell signal transduction in PC were activated, contributing to T cell depletion and creation of an inhibitory immune microenvironment in PC.

2.5. DDX60 knockdown impeded PC cell proliferation and metastasis in vitro

In addition to bioinformatics analysis, we also demonstrated the role of DDX60 in PC using cell experiments. We synthesized DDX60-specific siRNAs to inhibit endogenous DDX60 expression in BxPC-3 cells. The *DDX60* mRNA levels were reduced after siDDX60 transfection (Fig. 5A). The results of RT-qPCR were confirmed at the protein level using western blotting (Fig. 5B). The CCK-8 assay showed that *DDX60* knockdown significantly inhibited the proliferation of the PC cells (Fig. 5C). The transwell assay demonstrated that the migration and invasion ability of the PC cells decreased when transfected with the siRNA against DDX60 compared to that observed in the control group (Fig. 5D). In summary, these results indicated that DDX60 inhibited the development of PC in vitro.

3. Discussion

Traditional treatment strategies such as surgery, locoregional therapies, chemotherapy, and radiotherapy still provide low survival rates for most PC patients [9]. In recent years, tumor immunotherapy has provided either passive or active immunity against malignancies by

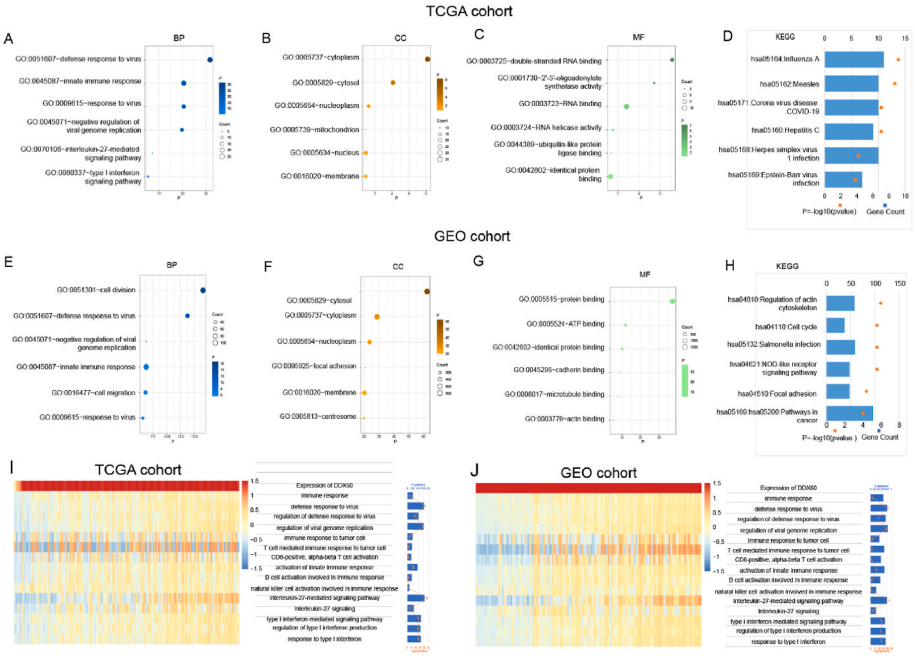

Fig. 3. Functional analysis of DDX60 in patients with pancreatic cancer. (A–C) The first six biological processes (BP), cellular components (CC), and molecular functions (MF) were most relevant to DDX60 in TCGA cohort. **(D)** KEGG pathway analysis of DDX60 in TCGA database. **(E–G)** The first six BP, CC, and MF were the most relevant to DDX60 in the Gene Expression Omnibus cohort. **(H)** KEGG pathway analysis of DDX60 in the GSE62452 dataset. **(I, J)** The heat map shows DDX60 expression and immune function enrichment scores in each patient in TCGA and GEO cohorts. The samples were arranged in ascending order of their DDX60 expression. The bar chart and line chart on the right show the R values and P values for the correlation analysis.

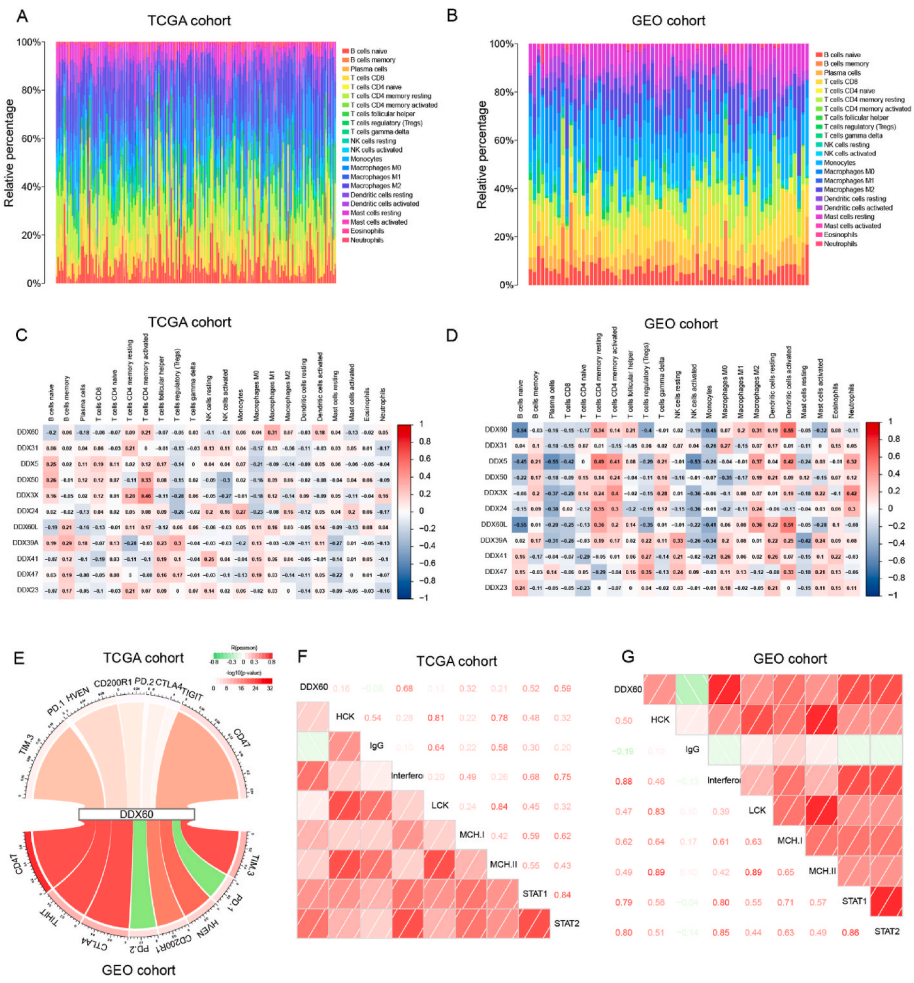

Fig. 4. DDX60 expression was related to the immune invasion of pancreatic cancer. (A, B) The heat map shows the proportion of immune cell infiltration in each PC sample in TCGA and GEO cohorts. **(C, D)** Pearson correlation of DDX family expression and tumor-associated immune cells in TCGA and GEO cohorts. **(E)** This circle shows the Pearson's association between DDX60 and inhibitory immune checkpoints. The inner fan color represents the R value. The color of the outer band represents the P value. Correlations were tested using Pearson correlation analysis. **(F, G)** The associated matrices of DDX60 and inflammation-related metagenes in TCGA and GEO cohorts, respectively. The correlation coefficients are shown in the upper right and lower left corners. The correlation coefficient can also be expressed as a rectangular shade. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

harnessing the immune system to target the tumors [31]. However, clinical trials of multiple immunotherapies and their combination with other treatments for PC have shown disappointing results. This article is mainly to explore the mechanism of immunosuppression in PC and find new targets for the treatment of PC.

Previous studies have shown that DDX60 is involved in the tumor progression of colorectal cancer [32]. DDX60 regulates the immune response of various viral diseases through a type I interference reaction and may promote the tumor immune escape of glioma [33]. According to the results of this study, DDX60 is highly expressed in PC tissues and associated with the poor prognosis. We identified DDX60 as a prognostic marker for PC. Functional enrichment analyses were conducted to further investigate the potential mechanism of action of DDX60. The results of KEGG pathway and GO analyses revealed significant enrichment of DDX60 in immune-related pathways. Results of GSVA further demonstrated that high DDX60 expression was significantly associated with multiple immune processes and regulation of inflammatory factors. Higher levels of infiltrating M0 macrophages, M2 macrophages, neutrophils, and Tregs in PC were significantly associated with shorter survival, whereas higher levels of tumor-infiltrating $CD4^+$ T cells, $CD8^+$ T cells, M1 macrophages, and dendritic cells were significantly associated with longer survival [34,35]. Inflammatory processes can affect all stages of tumor development, as well as therapy [36]. In the present study, we analyzed the correlation between DDX60 expression and tumor immunity from the perspective of immune cell infiltration, cancer immune checkpoint inhibitors, and inflammatory dysregulation in the tumor microenvironment. The results are similar to those of the inflammatory response at immune checkpoints and are consistent with the observations of previous studies [23]. Based on these results, we hypothesized that DDX60 in tumors interacts with tumor-infiltrating immune cells, leading to the upregulation of inhibitory immune checkpoints on the surface of immune cells, thereby rendering the tumor insensitive to immunotherapy, in addition, increased secretion of inflammatory factors also leads to inhibition of the tumor microenvironment and disease progression.

After analyzing the role of DDX60 in PC using bioinformatics methods, we further confirmed that DDX60 can influence the invasion and metastasis of PC using in vitro cell experiments. In this study, we demonstrated the prognostic significance of DDX60 in PC and its mechanism of action in PC cells, which are the novel features of this study. Our study confirmed the strong association between DDX60 and the immune microenvironment of PC, elucidating the mechanism of DDX60 action, and suggested that DDX60 may be a novel biomarker for immunotherapy. However, this study had some limitations. We are not currently developing new DDX60 inhibitors. Therefore, the effective targeting of DDX60 in patients with PC remains questionable.

Overall, we showed that DDX60 plays a vital role and may mediate an immunotherapeutic effect in PC. DDX60 is a predictive factor for the prognosis of PC and could be a new target for the treatment of PC. In future, we intend to demonstrate the role of DDX60 in the development of PC in animal models and dynamically observe changes in immune checkpoints and inflammatory cytokines to improve the accuracy of the study.

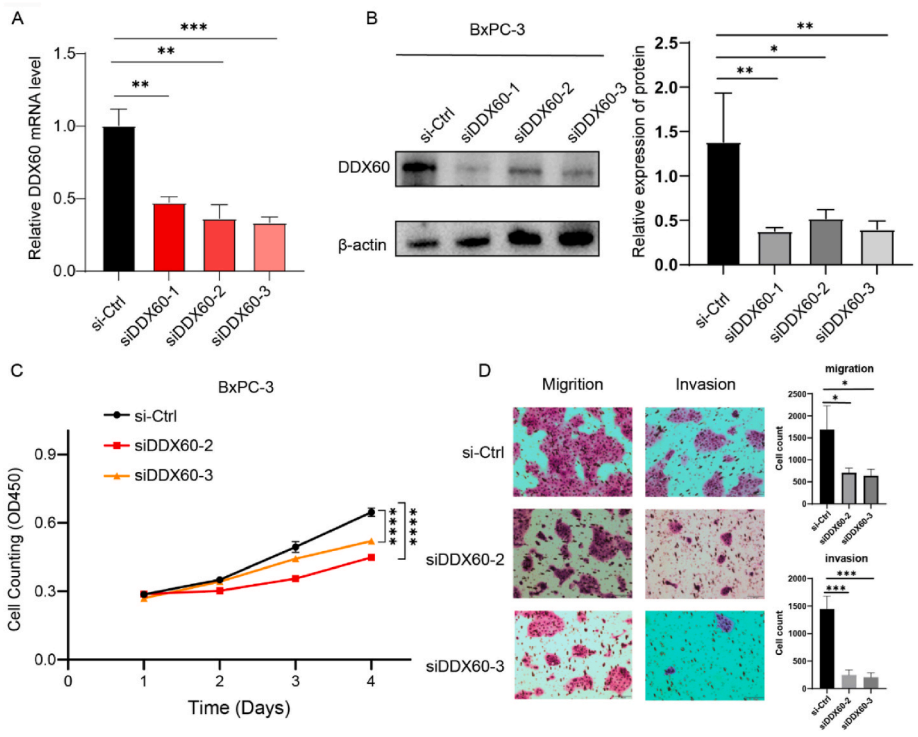

Fig. 5. DDX60 knockdown impeded PC cell proliferation and metastasis in vitro. (A, B) SiRNA-mediated DDX60 repression was confirmed using RT-qPCR and western blotting after RNA infection of BxPC-3 cells. **(C)** Inhibition of DDX60 impaired the growth of BxPC-3 cells. **(D)** Transwell assay was performed to measure the migration and invasion ability of BxPC-3 cells after transfection with siDDX60. Scale bar, 100 μm *P < 0.05, **P < 0.01, ***P < 0.001. ****P < 0.0001.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here:
http://tcga-data.nci.nih.gov/tcga/
http://www.ncbi.nlm.nih.gov/geo/
https://www.jianguoyun.com/p/DRPzxlYQk9qnCxjNw_oEIAA.

Author contributions

Hao Hu and Yong Mao conceived and designed the research. Yue Tao, Shuo Zhang and Leisheng Wang performed the experiments. Tiantian Lai, Xiaowen Su and Enhong Chen analyzed the data. Tiantian Lai and Xiaowen Su wrote the manuscript. All authors contributed to writing and critically revising the manuscript. All authors have read and approved to the published version of the manuscript.

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Declaration of competing interest

All authors declare that they have no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2023.101488.

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