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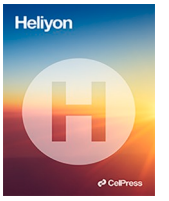

Research article

HMGA2 overexpression activates IGF2BP2 to stabilize APLP2 via m6A modification and promote pancreatic cancer progression

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ABSTRACT

Pancreatic cancer is a highly aggressive malignancy of the digestive system, with occult onset, rapid progression, and poor prognosis. The genetic heterogeneity of pancreatic cancer contributes to its highly malignant biological behavior. HMGA2 is overexpressed in tumors and is known to regulate tumor progression in various cancers through the HMGA2-IGF2BP2 axis, but its role and mechanism in pancreatic cancer remain unclear. In this study, we demonstrated that HMGA2 promotes pancreatic cancer progression. We further revealed that HMGA2 upregulates IGF2BP2, which stabilizes APLP2 mRNA via m6A modification, thereby promoting pancreatic cancer progression. These results indicate that HMGA2/IGF2BP2/APLP2 signaling axis regulates the progression of pancreatic cancer.

1. Introduction

Pancreatic cancer is a highly aggressive and malignant digestive system tumor with rapid progression. Currently, apart from radical surgery, the efficacy of other treatments is limited. The incidence of pancreatic cancer is increasing annually, and due to its insidious early symptoms, early detection is challenging. Patients with pancreatic cancer face a bleak prognosis and low five-year survival rate [1,2], as the disease has often already progressed or metastasized by the time of diagnosis. Consequently, it becomes crucial to identify novel therapeutic targets in order to enhance the chances of survival for individuals with this condition.

The high mobility group (HMG) domain is a DNA-binding motif with transcriptional regulatory functions. The high mobility group A2 (HMGA2) protein is a non-histone structural transcription factor that is involved in cell cycle, DNA damage repair, apoptosis and other cellular processes [3,4]. HMGA2 is overexpressed in various tumors and has been shown to facilitate tumor progression [5,6], but its role in pancreatic cancer remains controversial [7,8] and warrants further investigation.

Amyloid precursor protein (APP) is a type 1 transmembrane glycoprotein. Its homologs amyloid precursor-like protein 1 (APLP1) and amyloid precursor-like protein 2 (APLP2) are highly conserved in mammals. These molecules play a crucial role in the development and advancement of Alzheimer's disease, and they also display abnormal expression patterns in cancer cells [9]. Previous studies have reported that amyloid precursor-like protein 2 (APLP2) is abnormally upregulated in pancreatic cancer and promotes tumor progression [10,11]. We discovered that HMGA2 elevates APLP2 protein level in pancreatic cancer, and the underlying

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mechanism remains to be elucidated.

Insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) is an RNA-binding protein that regulates various biological functions. As an m6A reader, it participates in tumorigenesis and development by interacting with different RNAs [12,13]. It has been reported that IGF2BP2 is a target gene of HMGA2. HMGA2 overexpression can activate IGF2BP2 and exert its function in embryonal rhabdomyosarcoma and polycystic ovary syndrome [14,15]. However, whether this pathway contributes to tumor progression in pancreatic cancer remains to be explored.

Our study found that IGF2BP2 stabilizes APLP2 mRNA level through m6A modification. There exists a HMGA2-IGF2BP2-APLP2 signaling axis in pancreatic cancer that drives pancreatic cancer progression.

2. Methods

2.1. Cell lines and cell transfection

Pancreatic cancer cell lines (MIA PaCa-2, AsPC-1, MIA PaCa-2, PANC-1, BxPC-3) and pancreatic ductal epithelial cell (HPDE6-C7) were obtained from the Chinese Academy of Science Cell Bank. All the cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone, USA). The cells were maintained in a 37 °C incubator with 5% CO2. PANC-1 and BxPC-3 cells were seeded into 6-well plates at a density of 2.5×10^5 cells per well and incubated for 24 h before transfection. Transfection was performed using Lipofectamine 2000 (Thermo Fisher Scientific). The lentivirus-based control and gene-specific shRNAs (shHMGA2#1, shHMGA2#2, shIGF2BP2#1, shIGF2BP2#2, Sigma-Aldrich) were employed to interfere the expression of gene in 293T cells. Following transfection for 24 h, the transfection medium was replaced with DMEM, which had been prepared with 10% FBS and 1 mM sodium pyruvate. Subsequently, the virus culture solution was collected after 48 h of continuous culture and then mixed with PANC-1 and BxPC-3 cell lines supplemented with 12 μg/ml of polybrene. After incubating the mixture for 24 h, successfully infected cells were filtered using 10 μg/ml of puromycin. The specific sequence information for the shRNAs used in this study is provided in Supplementary Table S1.

2.2. Plasmids and reagents

Flag-HMGA2 plasmids were cloned into the CMV-MCS-3xFlag-SV40-neomycin vector by GENECHEM (Shanghai, China). In our research, we used the following primary antibodies: HMGA2 (20795-1-AP, proteintech; 1:5000 dilution), GAPDH (ab8245, Abcam; 1 : 5000 dilution), IGF2BP2 (11601-1-AP, proteintech; 1 : 2000 dilution), and APLP2 (20339-1-AP, proteintech; 1:1000 dilution).

2.3. Protein extraction and western blotting analysis

The cells were collected and lysed by utilizing a lysis buffer that consisted of 1% inhibitors for protease and phosphatase, and this process was carried out on ice for a duration of 15 min. A protein assay kit (Pierce Biotechnology, USA) was used to determine the protein concentration. Equal quantity of proteins was segregated within SDS-PAGE gels, and their detection was achieved by employing PVDF membranes after they were subjected to incubation with primary antibodies, followed by subsequent utilization of secondary antibodies.

2.4. Extraction of RNA and quantitative real-time PCR(qRT-PCR)

To extract total RNA from PANC-1 and BxPC-3 cells, we employed Trizol reagent (Thermo Fisher Scientific, USA). Subsequently, reverse transcription (PrimeScript™ RT reagent Kit) was performed to convert the total RNA into persistent cDNA. To amplify the cDNA, RT-PCR analysis was conducted using a PCR kit (TB Green™ Fast qPCR Mix). The normalization of all cycle indices was achieved by referencing GAPDH, and the 2-ΔCq method was utilized to quantify the multiple variations. Supplementary Table S2 contains the sequences of the forward and reverse primers.

2.5. RNA binding protein immunoprecipitation (RIP)

PANC-1 cells were cultured in 10-cm plates until they reached 80–90% confluence. Subsequently, the cells were accumulated and lysed with IP lysis buffer (G2038-100 ML, Servicebio, China), which was supplemented with a protease inhibitor (100 ×) and an RNase inhibitor (40 U/μl). This lysis process was carried out on ice for a duration of 30 min. The resultant cell lysates were then stored at − 80 °C for 5 min before being allowed to thaw on ice. To further purify the lysates, they were centrifuged at 12,000 g for 10 min. These cell lysates were divided into two parts: one serving as the input group for whole cell extraction, and the other utilized for subsequent immunoprecipitation (IP) treatment (IP group). Within the IP group, the cell lysates were alternately incubated with either 5 μg of *anti*-IGF2BP2(11601-1-AP, Proteintech; 1 : 2000 dilution) or IgG antibody (14,678–1-AP, Proteintech, Wuhan, China) throughout the night at 4 °C. To facilitate immunoprecipitation, protein A/G magnetic beads (Thermo Fisher Scientific) were washed five consecutive times with 0.1% Tween-20 in PBS. These beads were then mixed with the cell lysate-antibody complexes and rotated continuously at 4 °C for 6 h. Subsequently, the RNA-protein complexes were subjected to five consecutive rinses with elution buffer followed by treatment with proteinase K at 55 °C for 1 h. The bound RNAs were extracted and subjected to RT-qPCR for quantitative analysis. The primers for RT-qPCR were showed in Supplementary Table S2.

2.6. MeRIP-qPCR

The process of RNA extraction was conducted as previously described. A portion of 10% of the total RNA was set aside for the purpose of serving as the input control, while the remaining RNA samples were utilized for m6A-IP analysis. Magnetic beads were employed in conjunction with either the *anti*-m6A antibody (ab151230, abcam, USA) or IgG, employing the Dynabeads™ Antibody Coupling Kit (14311D, Invitrogen, USA) as per the guidelines set by the manufacturer. Next, the total RNA was mixed with the antibody-conjugated beads in a binding buffer volume of 500 μl and continuously rotated for a duration of 4 h at a temperature of 4 °C. The m6A-modified mRNAs were then extracted from the beads by applying elution buffer, which was followed by further purification in order to facilitate subsequent analysis through the use of RT-qPCR. The relative enrichment was normalized in relation to the input control. The primers for RT-qPCR were showed in Supplementary Table S2.

2.7. In vitro cell growth assay

1×10^4 pancreatic cancer cells were planted in 96-well plates, and the MTS solution (cat. no. ab197010; Abcam) was introduced following the instructions. Absorbance at 490 nm was measured to evaluate in vitro cell growth. For the process of colony creation evaluation, the cells were placed in 6-wells plates (500 cells/well) and incubated in a complete growth medium that contained 10% fetal bovine serum (FBS) at a temperature of 37 °C. After the course of 14 days, the cells underwent fixation in methanol for a duration of 30 min followed by staining using a 1% solution of Crystal Violet Staining Solution for another 30 min. Subsequently, the plates were rinsed with PBS three times. Finally, the calculation of the total number of colonies was performed.

2.8. In vitro cell invasion assay

The study employed Bio-Coat Matrigel (BD Biosciences, China) invasion chambers for in vitro cell invasion assays. Initially, cells

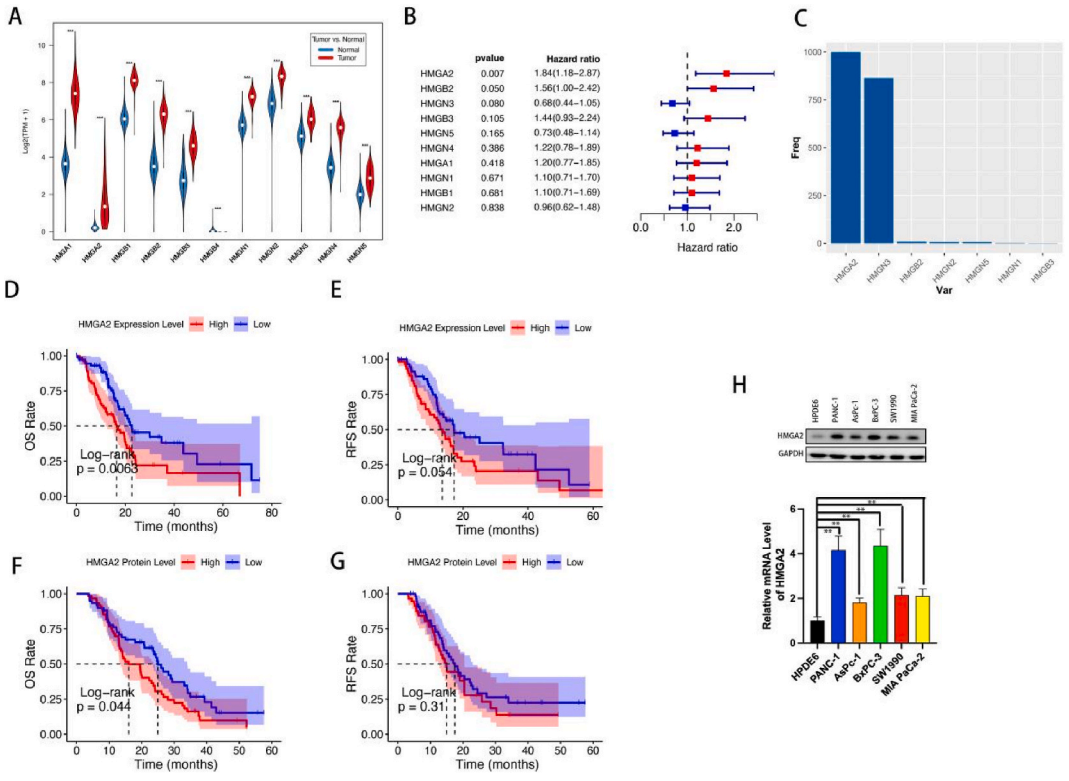

Fig. 1. HMGA2 Overexpression is Correlated with Poor Prognosis in Pancreatic Cancer. (A) mRNA expression levels of HMG family proteins in pancreatic cancer in the TCGA dataset. (B) COX regression analysis of HMG family proteins and pancreatic cancer RFS in the TCGA dataset. (C) Lasso-Cox analysis of HMG family proteins and pancreatic cancer RFS in the TCGA dataset. (D) Overall survival of high/low HMGA2 groups using TCGA dataset was analyzed, and p values are as indicated. (E) Disease-free survival of high/low HMGA2 groups using TCGA dataset was analyzed, and p values are as indicated. (F) Overall survival of high/low HMGA2 protein groups using CPTAC dataset was analyzed, and p values are as indicated. (G) Disease-free survival of high/low protein HMGA2 groups using CPTAC dataset was analyzed, and p values are as indicated.(H) Cells were obtained and used for western blotting analysis and RT-qPCR analysis. The data was exhibited as the mean accompanied by the standard deviation (SD), with three replicates (n = 3). The statistical significance of the results was determined using the one-way analysis of variance (ANOVA). **, $P < 0.01$.

were cultivated in the chamber inserts for 24 h and subsequently fixed using methanol for 15 min. Staining with crystal violet for 30 min was done to visualize the cells. The count of invading cells was conducted in a minimum of three fields per group.

2.9. In vivo tumor growth assay

Approval from the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology was obtained to ensure ethical standards in all animal experimentation conducted. BALB/c-nude mice aged between 4 and 5 weeks and weighing 18–20 g were procured from Shubeili (China). Standard living conditions were maintained for the mice, including a 12-h light/dark cycle, free access to food and water. To generate xenografts, PANC-1 cells were infected with lentiviral particles of varying types. Following puromycin selection for 48 h, 1×10^7 cells were subcutaneously injected into the mice's back. Measurements of xenografts' length and width were taken using a Vernier caliper, and tumor volumes were calculated using formula $(L \times W^2)/2$. At the termination

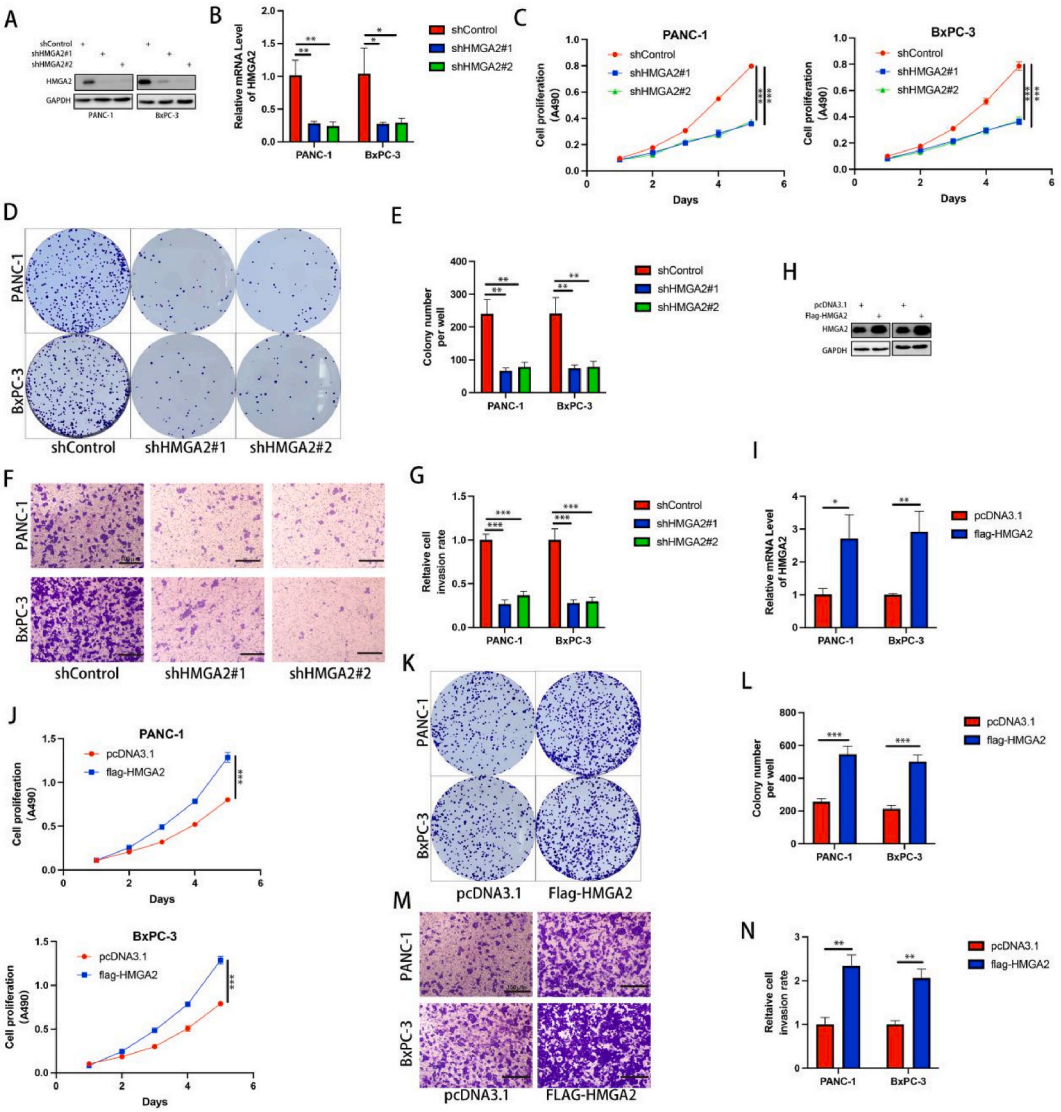

Fig. 2. HMGA2 Promotes Pancreatic Cancer Cell Progress. A-G) PANC-1 and BxPC-3 cells were infected with the indicated shRNA for 72 h. Cells were collected for western blotting analysis (A), RT-qPCR analysis (B), MTS analysis (C), colony formation assay (D–E), and Transwell analysis (F–G). For panels B, C, E, G, the data was exhibited as the mean accompanied by the standard deviation (SD), with three replicates (n = 3). The statistical significance of the results was determined using the one-way analysis of variance (ANOVA). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. H–N) PANC-1 and BxPC-3 cells were infected with the indicated plasmid for 48 h. Cells were collected for western blotting analysis (H), RT-qPCR analysis (I), colony formation assay (K–L), MTS analysis (J) and Transwell analysis (M − N). For panels J, L, N, the data was exhibited as the mean accompanied by the standard deviation (SD), with three replicates (n = 3). The statistical significance of the results was determined using the one-way analysis of variance (ANOVA). **, $P < 0.01$; ***, $P < 0.001$.

of the study, euthanasia was performed on the mice, and the tumors were excised and weighed.

2.10. Bioinformatics analysis

Bioinformatics analysis in this sutdy are sourced from the following databases: The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), CancerSEA, Clinical Proteomic Tumor Analysis Consortium (CPTAC), RNA Epitranscriptome Collection (REPIC) and ChIP-Atlas (Details in Supplementary Methods).

2.11. Statistical analysis

Statistical analysis in this study were conducted using the GraphPad Prism 9 software. Descriptive statistics are presented as means ± standard deviation (SD). The sample size (n) for each analysis can be found in the figure captions. Statistical significance was assessed using Student's t-test for comparisons between two groups and one-way or two-way ANOVA for comparisons among multiple groups. Results with a p-value less than 0.05 were deemed statistically significant.

3. Results

3.1. HMGA2 overexpression is correlated with poor prognosis in pancreatic cancer

High mobility group (HMG) family proteins play an important role in tumors [16]. In this study, we found that the HMG family proteins were highly expressed in pancreatic cancer in the TCGA database (Fig. 1 A). Through Lasso-Cox analysis, we found that HMGA2 had the closest relationship with pancreatic cancer survival, and HMGA2 high expression was associated with poor prognosis of pancreatic cancer [Fig. 1 B–C]. HMGA2 is overexpressed in various tumors and facilitates tumor progression [5,6]. Kaplan-Meier analysis showed that HMGA2 high mRNA expression and protein level led to reduced overall survival (OS) and recurrence-free survival (RFS) of pancreatic cancer patients [Fig. 1 D-G]. In addition, compared with non-malignant pancreatic ductal epithelial cells, HMGA2 expression level was elevated in pancreatic cancer cell lines (HPDE6; Fig. 1 H). These results suggest that HMGA2 may be a marker of poor prognosis in pancreatic cancer.

3.2. HMGA2 Promotes Pancreatic Cancer Cell Progress

Then, we examined the oncogenic function of HMGA2 in pancreatic cancer by silencing its expression using shRNA in PANC-1 and BxPC-3 pancreatic cancer cells (Fig. 2A–B); MTS analysis and colony formation assay showed that HMGA2 silencing significantly inhibited the proliferative ability of cancer cells (Fig. 2C–E). In addition, transwell assay revealed that HMGA2 knockdown significantly downregulated the invasive ability of cancer cells (Fig. 2F–G). Conversely, we found that HMGA2 overexpression (Fig. 2H–J)

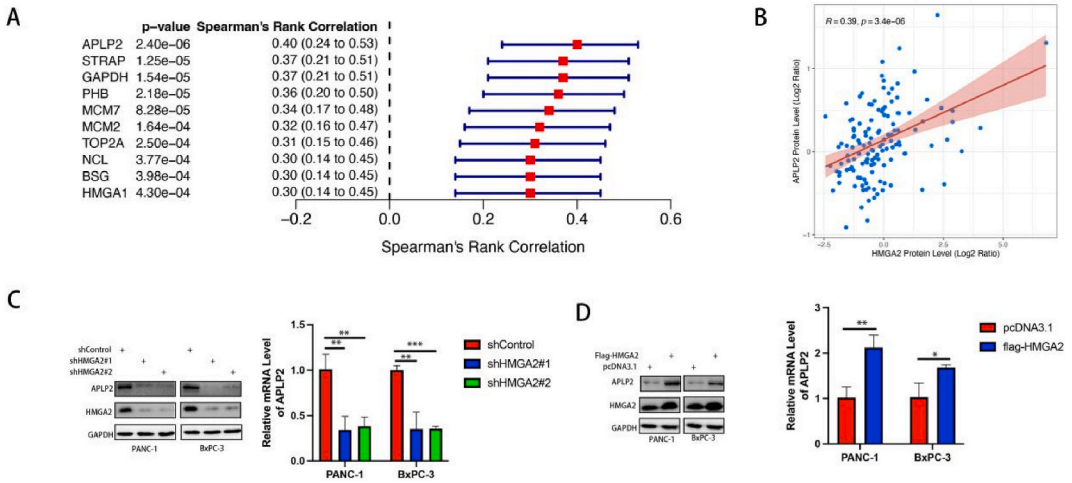

Fig. 3. HMGA2 Activates APLP2 in Pancreatic Cancer. (A) Using the CPTAC dataset to analyze the genes correlated with HMGA2 expression level, the correlation R and p values are shown in figure. (B) Using the CPTAC database to analyze the correlation between HMGA2 and APLP2 protein levels, the correlation R and p values are shown in the figure.(C)PANC-1 and BxPC-3 cells were infected with the indicated shRNA for 72 h. Cells were collected for western blotting analysis and RT-qPCR analysis, RT-qPCR data was exhibited as the mean accompanied by the standard deviation (SD), with three replicates (n = 3). The statistical significance of the results was determined using the one-way analysis of variance (ANOVA). **, P < 0.01; ***, P < 0.001. (D) PANC-1 and BxPC-3 cells were infected with the indicated plasmid for 48 h. Cells were collected for western blotting analysis and RT-qPCR analysis, RT-qPCR data was exhibited as the mean accompanied by the standard deviation (SD), with three replicates (n = 3). The statistical significance of the results was determined using the one-way analysis of variance (ANOVA). *, P < 0.05; **, P < 0.01.

enhanced the proliferation and invasion abilities of PANC-1 and BxPC-3 cells (Fig. 2K-N). These in vitro cell experiments collectively demonstrate that HMGA2 plays a critical role in the progression of pancreatic cancer.

3.3. HMGA2 activates APLP2 in pancreatic cancer

To elucidate the mechanism of HMGA2 regulates the progression of pancreatic cancer, we analyzed the proteins that exhibit the

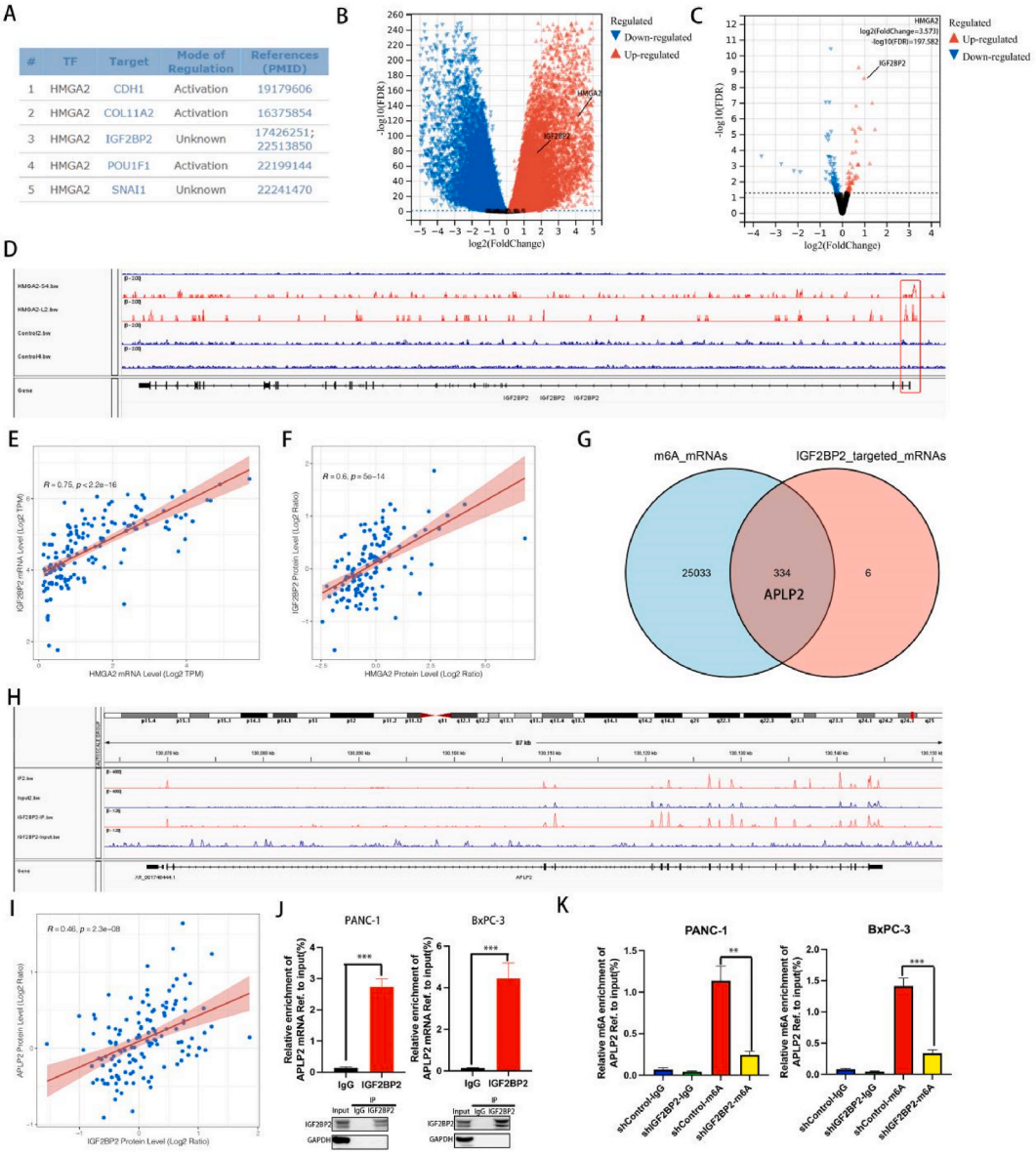

Fig. 4. HMGA2 activates IGF2BP2 to stabilize APLP2 via m6A modification. (A) TRRUST database screening for HMGA2 transcriptional target genes. (B) Volcano Plot analysis of gene differential expression in pancreatic cancer tissues in TCGA database, log2(FoldChange) and -log10(FDR) are shown in figure. (C) Volcano Plot analysis of gene differential expression in $CD34^{+}$ cells (GSE107594), gene log2(FoldChange) and -log10(FDR) are shown in figure. (D) ChIP Atalas was used to display HMGA2 ChIP-seq at IGF2BP2 gene locus. (E) Using the TCGA database to analyze the correlation between IGF2BP2 and HMGA2 mRNA levels, the correlation R and p values are shown in figure. (F) Using the CPTAC database to analyze the correlation between IGF2BP2 and APLP2 protein levels, the correlation R and p values are shown in figure. (G) Veen diagram showed that the target genes of m6A and IGF2BP2 in REPIC database. (H) REPIC database was used to display IGF2BP2 m6A RIP-seq at APLP2 gene locus. (I) Using the CPTAC database to analyze the correlation between IGF2BP2 and APLP2 protein levels, the correlation R and p values are shown in figure. (J) The RIP-qPCR analysis demonstrated the increased presence of APLP2 mRNA in the precipitates of *anti*-IGF2BP2. ***P < 0.001. Western blotting was conducted using PANC-1 and BxPC-3 cells. (K) MeRIP-qPCR analysis was performed to evaluate the m6A enrichment of APLP2 mRNA in PANC-1 and BxPC-3 cells after silencing IGF2BP2, using *anti*-IgG and *anti*-m6A antibodies. **P < 0.01 and ***P < 0.001.

highest correlation with HMGA2 in the CPTAC database and found that APLP2 had the closest relationship with HMGA2 protein level, and HMGA2 and APLP2 protein levels were significantly positively correlated (Fig. 3A–B). Previous studies have reported that amyloid precursor-like protein 2 (APLP2) is abnormally upregulated in pancreatic cancer and promotes tumor progression [10,11]. Further experiments showed that HMGA2 knockdown reduced the protein level and mRNA level of APLP2 (Fig. 3C), while HMGA2 overexpression upregulated the protein level and mRNA level of APLP2 (Fig. 3D). Therefore, the above results indicated that HMGA2 may influence pancreatic cancer progress by activating APLP2.

3.4. HMGA2 activates IGF2BP2 to stabilize APLP2 via m6A modification

To explore the mechanism of HMGA2 activating APLP2 in pancreatic cancer cells, we found that HMGA2 was a transcription factor of IGF2BP2 (Fig. 4 A). GEO database analysis of tissues and cells found that HMGA2 mRNA expression and IGF2BP2 mRNA expression were simultaneously upregulated (Fig. 4B–C). We analyzed publicly available HMGA2 ChIP-seq data and found that there was a significant HMGA2 binding peak in the gene promoter region of IGF2BP2 (Fig. 4 D). It has been reported that HMGA2 overexpression can activate IGF2BP2 via the transcriptional pathway [14,15]. Through TCGA database and CPTAC database we found that the mRNA and protein level of HMGA2 and IGF2BP2 were significantly positively correlated [Fig. 4 E-F]. We further investigated whether HMGA2 regulates APLP2 through IGF2BP2. IGF2BP2 is an m6A reader, mainly stabilizing mRNA levels by binding to them. By screening the potential m6A-modified target genes and the target genes of IGF2BP2, the Venn diagram shows that APLP2 may be regulated by IGF2BP2 through m6A modification [Fig. 4 G]. We analyzed publicly available IGF2BP2 RIP-seq data and found that there were significant IGF2BP2 m6A modification binding peak in APLP2 [Fig. 4 H]. Through CPTAC database we found that the protein level of IGF2BP2 and APLP2 were significantly positively correlated [Fig. 4 I].

Utilizing the IGF2BP2 antibody, we conducted RIP-qPCR experiments in PANC-1 and BxPC-3 cells. Compared to the IgG control group, the outcome exhibited a notable enrichment of APLP2 mRNA (Fig. 4 J). The results showed the interaction between APLP2 mRNA and IGF2BP2. To validate the impact of m6A modification on APLP2, we performed MeRIP-qPCR assays and observed that depletion of IGF2BP2 significantly diminished the m6A levels of APLP2 in PANC-1 and BxPC-3 cells (Fig. 4 K), contrasting with the corresponding control cells. In summary, our data suggest that in pancreatic cancer, HMGA2 overexpression activates IGF2BP2, which stabilizes APLP2 mRNA expression by m6A modification.

3.5. HMGA2-IGF2BP2-APLP2 axis promotes pancreatic cancer progression

To verify the HMGA2-IGF2BP2-APLP2 axis and its role in the progression of pancreatic cancer, we knocked down HMGA2 in pancreatic cancer cells, and APLP2 expression decreased, while knocking down IGF2BP2 enhanced this effect (Fig. 5 A). On the other hand, overexpressing HMGA2 increased APLP2 expression, and knocking down IGF2BP2 inhibited this phenomenon (Fig. 5 B). We

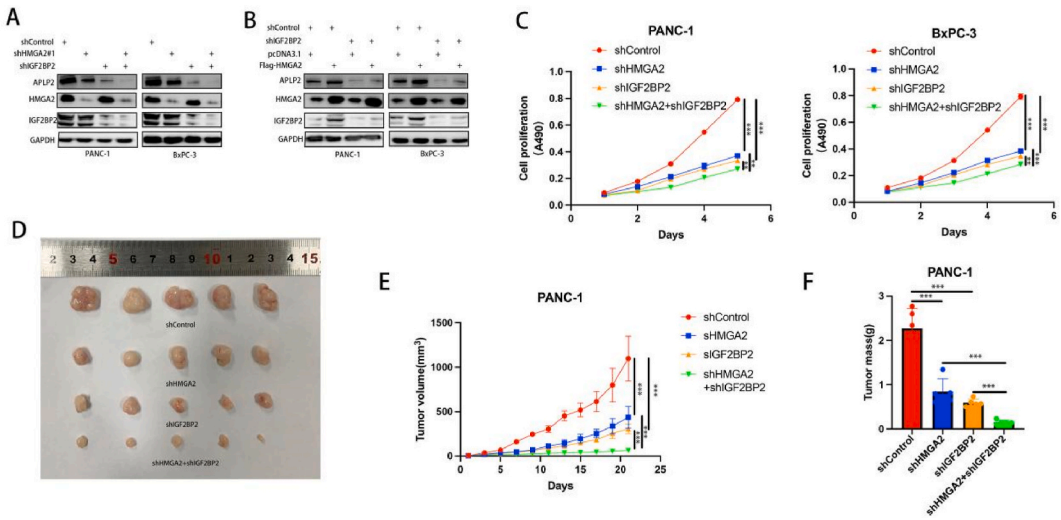

Fig. 5. HMGA2-IGF2BP2-APLP2 axis promotes pancreatic cancer progression. (A) PANC-1 and BxPC-3 cells were infected with indicated shRNA for 72 h. Cells were harvested for Western blot analysis. (B) PANC-1 and BxPC-3 cells were infected with indicated shRNA and plasmid for 48 h. Cells were harvested for Western blot analysis. (C) PANC-1 and BxPC-3 cells were infected with indicated shRNA for 72 h, cells were harvested for MTS analysis, data was exhibited as the mean accompanied by the standard deviation (SD), with three replicates (n = 3). The statistical significance of the results was determined using the one-way ANOVA. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (D–F) In vivo animal experiments. PANC-1 cells were infected with indicated shRNA for 72 h, after puromycin selection, cells were harvested and injected into nude mice subcutaneously for xenograft experiment, tumor images (D), tumor mass (E), tumor growth curve (F). Data was exhibited as the mean accompanied by the standard deviation (SD), with three replicates (n = 3). The statistical significance of the results was determined using the Student's *t*-test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

further examined the effect of HMGA2 and IGF2BP2 silencing on pancreatic cancer cell proliferation in vitro, and found that HMGA2 and IGF2BP2 silencing significantly inhibited pancreatic cancer cell proliferation ability in vitro by MTS analysis (Fig. 5C). Further in vivo experiments, we subcutaneously injected PANC-1 cells (shControl; shHMGA2; shIGF2BP2; shHMGA2+shIGF2BP2) into nude mice and evaluated tumor growth (Fig. 5D–F). We found that combined silencing of HMGA2 and IGF2BP2 further inhibited pancreatic cancer proliferation in vivo.

4. Discussion

High mobility group (HMG) proteins are non-histone, chromatin-associated molecules that participate in the maintenance and regulation of DNA, including replication, recombination, transcription and DNA repair processes. This superfamily of proteins mainly comprises HMGA, HMGB and HMGN, which are involved in transcriptional regulation and maintaining chromatin structure [16]. Our research indicates that among the HMG family, HMGA2 was most closely associated with the survival of pancreatic cancer. HMGA2 is overexpressed in a wide range of tumors and facilitates tumor progression, including lung cancer, breast cancer and ovarian cancer [17–19]. HMGA2 has a long 3′UTR, which provides a long targeting region for different miRNAs. The let-7 family of miRNAs was the first reported miRNA to regulate HMGA2 [20], and numerous miRNAs have been identified as involved in HMGA2 regulation [3]. The role of HMGA2 in pancreatic cancer is controversial. Studies have shown that patients with pancreatic cancer with high expression of the OLR1-*c*-Myc-HMGA2 axis had worse prognosis and enhanced pancreatic cancer metastasis [8]. Conversely, research in mice suggested that Hmga2 could serve as a prognostic marker for pancreatic cancer, but had limited impact on PDAC progression and treatment resistance [7]. We observed that HMGA2 was elevated in pancreatic cancer cell lines and promoted their proliferation and invasion abilities. The underlying mechanism require further investigation.

Literature reports indicate that amyloid precursor-like protein 2 (APLP2) is highly expressed in pancreatic cancer and influences the actin cytoskeleton and promoting pancreatic cancer progression [10]. Our findings demonstrate that HMGA2 was positively correlated with APLP2 protein level, and HMGA2 overexpression upregulated APLP2 expression. Whether HMGA2 overexpression promotes pancreatic cancer progression by upregulating APLP2 and its specific mechanism remains to be further investigated.

N6-methyladenosine (m6A) is the most abundant internal modification of RNA in eukaryotic cells and is a current research hotspot. M6A modification affects RNA metabolism from multiple pathways and plays an important role in cancer [21]. Insulin-like growth factor-2 mRNA-binding proteins 1, 2 and 3 (IGF2BP1, IGF2BP2, IGF2BP3) belong to a conserved family of RNA-binding oncogenic proteins that are m6A readers. Their expression is related to various levels, but more studies focus on IGF2BP1 and IGF2BP3 [12]. Human insulin-like growth factor 2 (IGF2) mRNA-binding protein 2 (IGF2BP2/IMP2) is an RNA-binding protein that regulates various biological processes [13]. It was previously considered to be a type 2 diabetes-related gene and is associated with various metabolic diseases, including diabetes, obesity, fatty liver, etc [22,23]. As an m6A reader, IGF2BP2 typically contributes to the stabilization of target gene RNA and plays a facilitating role in various tumors [13,23]. In colon cancer, IGF2BP2 stabilizes CREB1 mRNA through m6A modification and promotes colon cancer progression [24]. Although previous studies have identified Previous studies have reported that there exists a HMGA2-IGF2BP2 axis in various diseases. HMGA2 overexpression can activate IGF2BP2, including polycystic ovary syndrome and embryonal rhabdomyosarcoma [14,15], but whether there exists a HMGA2-IGF2BP2-APLP2 axis that promotes tumor progression in pancreatic cancer is unclear. Our study shows that HMGA2 was positively correlated with IGF2BP2 protein level and HMGA2 could promote IGF2BP2 transcription. Further studies revealed that as an m6A recognition factor, IGF2BP2 could bind to APLP2 mRNA and stabilize its level through m6A modification.

The oncogenic KRAS mutation is one of the most prevalent mutations in pancreatic cancer [25]. Studies have shown that IGF2BP2 is strongly associated with cancer metastasis and the expression of oncogenic factor KRAS [12]. In addition, APLP2 expression is significantly increased in the KRAS mutant mouse model [11]. Therefore, HMGA-IGF2BP2-APLP2 signaling axis may be activated KRAS mutations in pancreatic cancer, although this hypothesis requires further experimental validation.

In summary, our study demonstrates that we found that HMGA2 overexpression was associated with pancreatic cancer progression and poor prognosis. We have identified a HMGA-IGF2BP2-APLP2 signaling axis in pancreatic cancer that drives its malignant biological behavior. Our study might provides novel therapeutic targets for pancreatic cancer.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors (394232374@qq.com) on reasonable request.

Ethical approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki principles. It was approved by the Animal Use and Care Committees at Tongji Medical College, Huazhong University of Science and Technology ([2022] Approval IACUC Number:3546).

CRediT authorship contribution statement

Ke Liu: Writing – original draft, Conceptualization. **Congbing Wei:** Supervision, Conceptualization. **Haixin Yu:** Methodology, Funding acquisition, Data curation, Conceptualization. **Qun Zhang:** Resources, Investigation. **Zhouyuan Du:** Writing – review &

editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27268.

Abbreviations

APLP2	amyloid precursor-like protein 2
HMGA2	The high mobility group A2
IGF2BP2	Insulin-like growth factor 2 mRNA-binding protein 2
RIP	RNA binding protein immunoprecipitation
MeRIP	Methylated RNA Immunoprecipitation

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