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ABOUT COVER

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The primary aim of *World Journal of Gastrointestinal Oncology* (*WJGO*, *World J Gastrointest Oncol*) is to provide scholars and readers from various fields of gastrointestinal oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJGO mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal oncology and covering a wide range of topics including liver cell adenoma, gastric neoplasms, appendiceal neoplasms, biliary tract neoplasms, hepatocellular carcinoma, pancreatic carcinoma, cecal neoplasms, colonic neoplasms, colorectal neoplasms, duodenal neoplasms, esophageal neoplasms, gallbladder neoplasms, *etc.*

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Basic Study

Genome-wide CRISPR-Cas9 screening identifies that hypoxia-inducible factor-1 α -induced CBX8 transcription promotes pancreatic cancer progression via IRS1/AKT axis

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Abstract

BACKGROUND

Pancreatic cancer (PC) is one of the most lethal malignancies worldwide. It is known that the proliferation of PC cells is a critical process in the disease. Previous studies have failed to identify the key genes associated with PC cell proliferation, using bioinformatic analysis, genome-wide association studies, and candidate gene testing.

AIM

To investigate the function of the chromobox 8 (CBX8)/receptor substrate 1 (IRS1)/AKT axis in PC.

METHODS

A genome-wide CRISPR-Cas9 screening was performed to select genes that could facilitate PC cell proliferation. Quantitative reverse transcription-polymerase chain reaction was used to detect the expression of CBX8 in PC tissues and cells. The regulatory roles of CBX8 in cell proliferation, migration, and invasion were verified by *in vivo* and *in vitro* functional assays.

RESULTS

CBX8 was upregulated in PC tissues and shown to drive PC cell proliferation.

interest for this work.

Data sharing statement: No additional data are available.

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Higher expression of CBX8 was correlated with worse outcomes of PC patients from two independent cohorts comprising a total of 116 cases. CBX8 was also proved to serve as a promising therapeutic target for a PC xenograft model. We demonstrated that hypoxia-inducible factor (HIF)-1 α induced CBX8 transcription by binding to the promoter of *CBX8*. CBX8 efficiently activated the PI3K/AKT signaling by upregulating insulin IRS1.

CONCLUSION

CBX8 is a key gene regulated by HIF-1 α , and activates the IRS1/AKT pathway, which suggests that targeting CBX8 may be a promising therapeutic strategy for PC.

Key Words: CRISPR-Cas9 screening; Pancreatic cancer; Chromobox 8; Hypoxia; pI3K/AKT signaling

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Core Tip: The authors demonstrated that hypoxia-inducible factor-1 α induced chromobox (CBX)8 transcription by binding to the promoter of *CBX8*. CBX8 efficiently activated the PI3K/AKT signaling by upregulating insulin receptor substrate 1. The newly identified signaling axis may support the development of new therapeutic strategies for pancreatic cancer.

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INTRODUCTION

Pancreatic cancer (PC) is one of the most lethal cancers worldwide. It has become the second most fatal cancer in the United States. The 5-year survival rate of pancreatic ductal adenocarcinoma (PDAC) is < 10% due to late diagnosis and resistance to systemic therapies[1]. Many studies have shown that the prognosis of patients with PC remains poor after complete surgical resection. At the same time, due to the difficulty of diagnosis, many patients with PC have no chance of surgery after diagnosis. Therefore, it is important to study the occurrence and development of PC itself and the corresponding targeted therapy.

Hypoxia is one of the important characteristics of PC[2]. Activation of hypoxia-inducible factors (HIFs; particularly HIF-1 α and HIF-2 α) is an important mechanism for tumor cells to adapt to a hypoxic microenvironment. Our previous studies indicated that HIFs not only regulate the growth and metastasis of PC cells, but also mediate the immunosuppression[3] and angiogenesis[4] in PC.

Chromobox 8 (CBX8) (also known as human polyclonal 3), together with CBX2, CBX4, CBX6, and CBX7, are members of the CBX protein family. CBX8 plays an oncogenic role in different types of cancer. Zhang *et al*[5] reported that CBX8 upregulates Leucine Rich Repeat Containing G Protein-Coupled Receptor 5 (LGR5), leading to increased stemness and decreased chemosensitivity of colon cancer cells. CBX8 promotes tumor growth and metastasis in hepatocellular carcinoma (HCC)[6] and breast cancer[7]. However, whether CBX8 is involved in the proliferation of PC cells remains unknown.

Genome-scale CRISPR-Cas9 knockout (GeCKO) library is a powerful tool for the assessment of gene function[8] and the screening for genes involved in cancer cell proliferation and metastasis. In this study, Bxpc-3 and PANC1 cells were transduced with library lentiviruses. The cells were then injected subcutaneously into nude mice and removed 21 d later to sequence the single guide RNA (sgRNA) in the tumor tissue. Among all candidate genes, *CBX8* was selected for further analysis because of

its upregulation in cells. Furthermore, higher CBX8 expression was correlated with worse clinical outcomes of PDAC patients from two independent cohorts. We also showed that CBX8 was a key gene that was regulated by HIF-1 α , and could activate the IRS1/AKT pathway. The above findings suggest that targeting CBX8 may be a promising therapeutic strategy for PC.

MATERIALS AND METHODS

Cell culture and reagents

PC cell lines (PANC1 and Bxpc-3) and 293T cells were purchased from American Type Culture Collection (Manassas, VA, United States) and maintained in RPMI-1640 medium containing 10% fetal bovine serum and 1% penicillin/streptomycin in a 95% air/5% CO₂ environment at 37 °C. Cells at passages 3–15 were used in this study. For hypoxic culture, cells were cultured under 1% O₂.

Lentiviral packaging and infection

The Human CRISPR Knockout Pooled Library was obtained from Addgene (<http://www.addgene.org/crispr/Libraries/geckov2/>, United States). GeCKO library plasmids, pVSVg (AddGene) and psPAX2 (AddGene), were added to 100 μ L Opti-MEM at a ratio of 1:0.5:1.5. After 15-min incubation with Lipofectamine 3000 (Invitrogen, Carlsbad, CA, United States), the mixture was added to 293T cells. Forty-eight hours later, cell supernatants containing lentiviruses were collected. PANC1 and BXP-3 cells were transduced at a low multiplicity of infection (0.3) to ensure that most cells received only one viral construct. The cells were selected with puromycin (1 μ g/mL) for 14 d. Only cells transduced with a LentiCRISPR construct could survive. After transfection with GeCKO library, cells were transplanted subcutaneously into the right flank of 4-wk-old male nude mice. Twenty-one days later, the mice were killed and primary tumors were removed for sgRNA sequencing.

Cytotoxicity assay

Following infection, cells were treated with gemcitabine for 48 h. Cell viability was assessed by CCK-8 assay (Beyotime, China) and the IC₅₀ values were calculated using GraphPad Prism 7 (GraphPad, La Jolla, CA, United States). All measurements were performed in triplicate. The abundance of sgRNA was determined by deep sequencing.

Colony formation assay

Cells were treated with DMSO or chemotherapeutic agents for 24 h and then seeded in six-well plates (200/well). The cultures were maintained in a 5% CO₂ incubator at 37 °C for 2 wk. The colonies were fixed with 4% paraformaldehyde, followed by staining with 0.5% crystal violet. The number of colonies in each group was counted under a microscope. Independent experiments were performed in triplicate.

Luciferase activity assay

The promoter regions of CBX8 and IRS1 were amplified by polymerase chain reaction (PCR) and cloned into RB reporter plasmids (Ribo, China). CBX8 knockdown plasmids were cotransfected into 293T cells. Twenty-four hours later, cells were harvested and Renilla and firefly luciferase activities were detected using a dual-luciferase reporter assay kit. The Renilla luciferase activity was used for normalization.

Chromatin immunoprecipitation and chromatin immunoprecipitation sequencing

A chromatin immunoprecipitation (ChIP) assay was carried out using an EZ-ChIP Chromatin Immunoprecipitation Kit (Millipore, Bedford, MA, United States). Formaldehyde (1%) was used to crosslink proteins and DNA for 10 min. Cell lysates were sonicated to obtain DNA fragments, which were subjected to IP with primary antibodies or negative control IgG. Purified DNA was analyzed by quantitative reverse transcription PCR (qRT-PCR) with SYBR Green Master Mix (Promega, Beijing, China). The relative enrichment values were calculated through normalization of the results to the input values and are expressed relative to the values obtained with normal IgG.

In vivo experiment

All animal experimental procedures were approved by the Ethics Committee for

Animal Research of Shanghai Jiaotong University School of Medicine (Shanghai, China). Four-week-old male nude mice were subcutaneously transplanted in the right flank with CBX8-silenced or control PC cells (PANC1, Bxpc-3, 2×10^6 cells per mouse) in 100 μ L PBS mixed 1:1 with BD Matrigel Basement Membrane Matrix (Corning, Corning, NY, United States) as previously reported. Tumor size was measured every 3 d with a digital caliper. When tumor volume reached approximately 100 mm³ (day 6 postinoculation), mice were randomly assigned to each group. After 21 d, the tumor weight was detected and mice were killed.

Patients' samples

This study was approved by the Human Research Ethics Committee of Shanghai General Hospital (Shanghai, China) and performed following the United States Common Rule. Each patient provided written informed consent. A total of 116 archived PDAC specimens were collected. Patients were followed over time.

Statistical analysis

All data are shown as the mean \pm SD. Statistical analyses were performed using Graphpad Prism 7. Student's *t* test or analysis of variance was used to compare continuous variables.

RESULTS

Genome-wide pooled sgRNA library screening identifies the genes affecting the growth of PC cells in vivo

To identify the genes responsible for growth of PC cells, we transduced PANC1 and Bxpc-3 cells with GeCKO library lentiviruses. After 14 d of puromycin selection, cells were transplanted subcutaneously into the right flank of 4-wk-old male nude mice. Twenty-one days later, the mice were killed and primary tumors were removed for sgRNA sequencing (Figure 1A). About 1.6×10^6 sgRNA sequences were obtained from each tumor sample. Genomic DNA was extracted from tumor tissues for PCR and next-generation sequencing (NGS) analysis. The expression of genes in each sample was measured. The genes were ranked according to the numbers of sgRNA and NGS reads. Compared to the control group, 872 cell growth related candidate genes were identified in PANC1 cells, while 819 were found in Bxpc-3 cells. There were 244 genes identified in both groups with differentially enriched sgRNA. We performed qPCR to confirm the candidate top 10 differently expressed sgRNAs from NGS analysis (Figure 1B), which indicated that *CBX8* had the lowest expression level of sgRNA (Figure 1C). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and gene set enrichment analysis (GSEA) showed that these universal differentially expressed sgRNAs were enriched in the PI3K/AKT pathway (Figure 1D and E).

Higher CBX8 expression induces worse clinical outcome in PC patients

To determine whether *CBX8* is involved in PC progression, we examined the mRNA level of *CBX8* in 116 paired PC and adjacent nontumor tissue samples and found that *CBX8* expression was significantly increased in PC tissues compared with corresponding noncancer tissues (Figure 2A). Kaplan-Meier survival analysis indicated that patients with higher *CBX8* expression had a shorter overall survival than those with low *CBX8* expression (Figure 2B). The protein content of *CBX8* in these tissue samples was obtained by Western blot analysis (Figure 2C). Compared to normal neighboring tissues, the *CBX8* protein content in PC tissues was increased.

We analyzed the expression of *CBX8* in The Cancer Genome Atlas (TCGA) database to confirm its potential regulatory pathways and cellular functions. KEGG pathway analysis found that genes positively correlated to *CBX8* (Figure 2D) were enriched in the PI3K/AKT and pancreatic adenocarcinoma pathway (Figure 2E).

CBX8 is required for efficient proliferation of PC cells in vitro and in vivo

We measured the protein level of *CBX8* in PC cell lines and found that *CBX8* was upregulated in PANC1 and Bxpc-3 cells compared with normal pancreatic epithelial cells (Figure 3A). CCK-8 assay was used to assess the effect of *CBX8* on PC cell proliferation, which indicated that *CBX8* knockdown (Figure 3B) reduced the proliferation of PC cells (Figure 3C and D). Colony formation assay revealed that *CBX8* silencing impeded the proliferation of PC cells (Figure 3E).

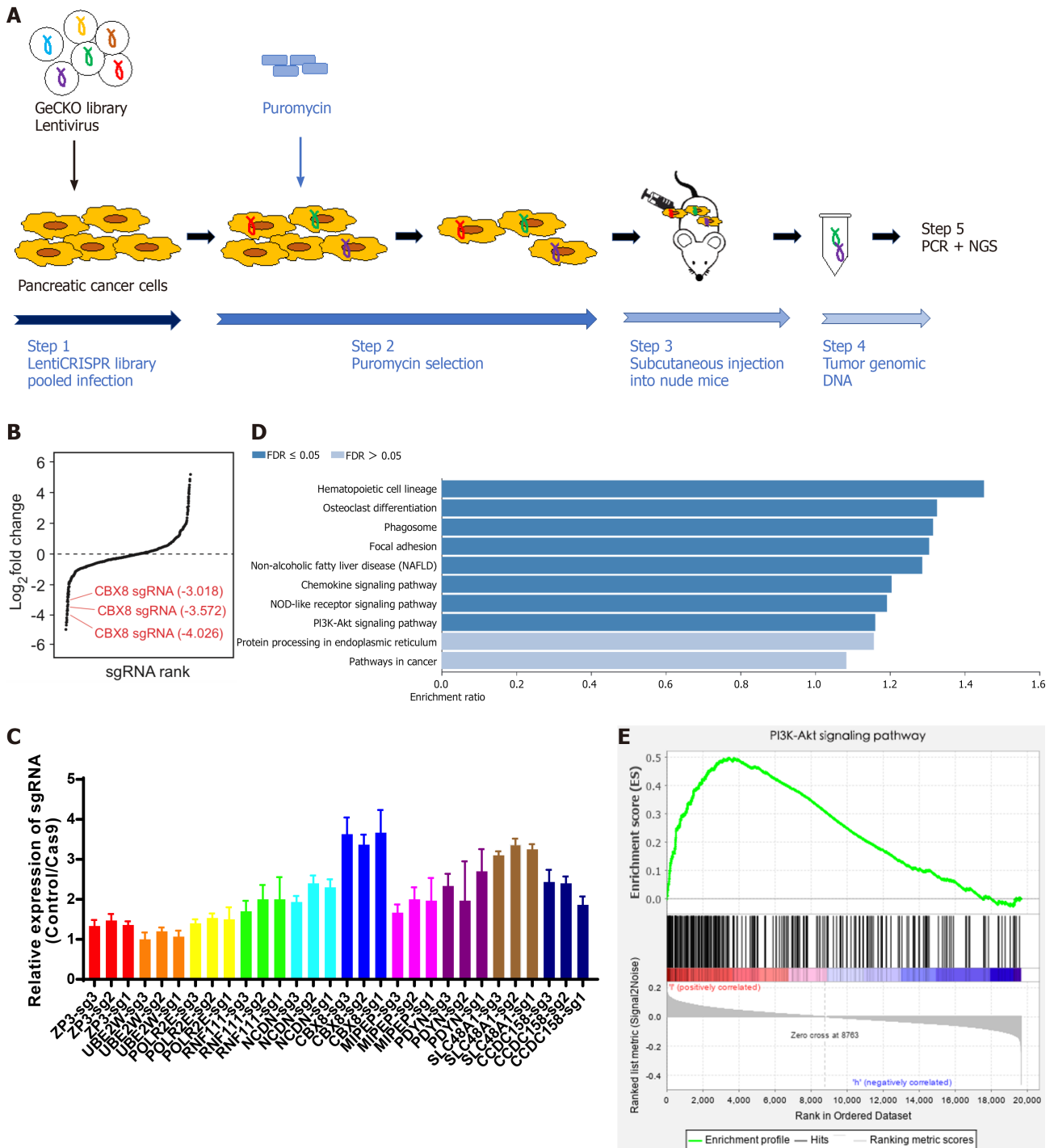
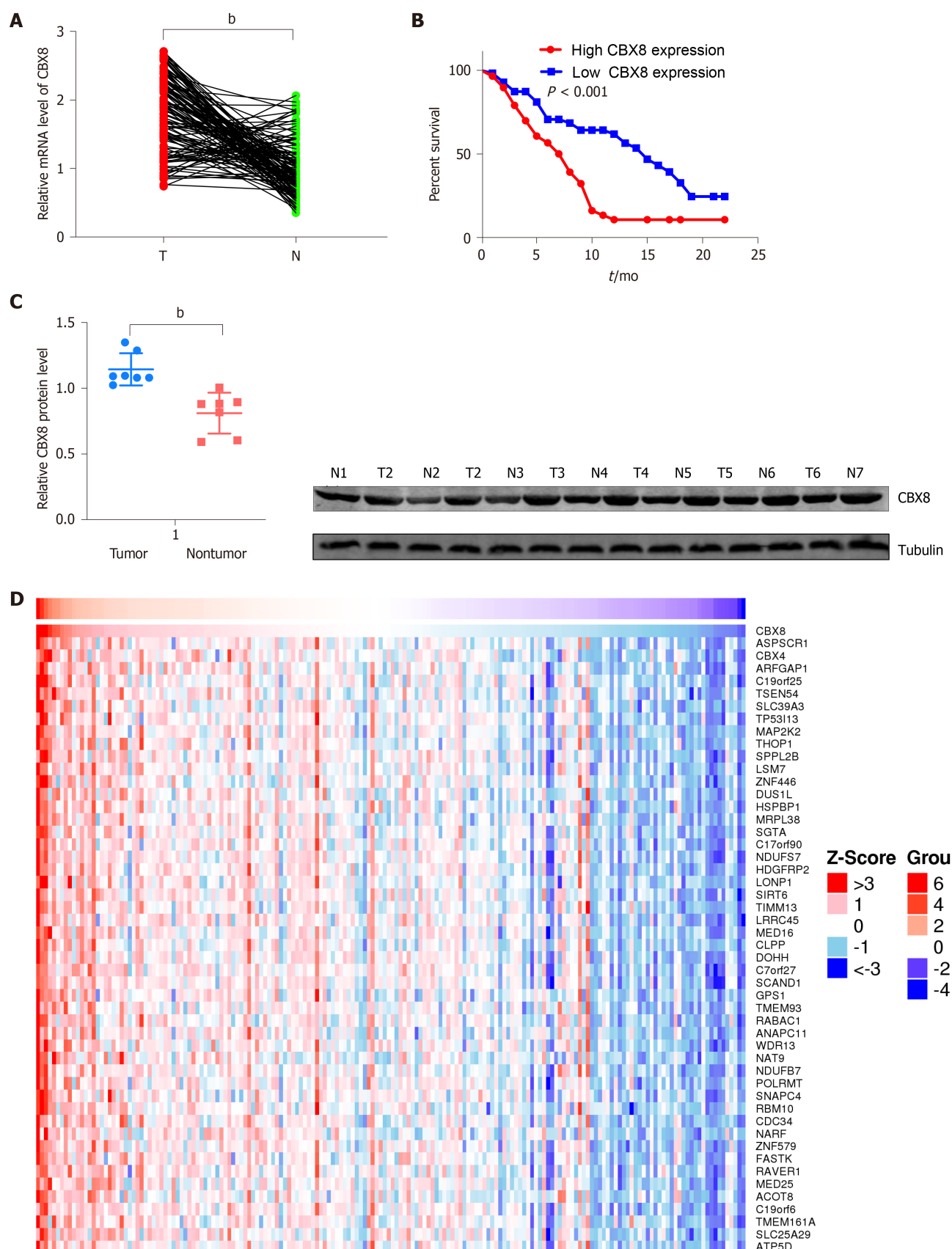


Figure 1 Genome-wide pooled sgRNA library screening identifies genes affecting growth of pancreatic cancer cells *in vivo*. A: Schematic overview of experimental timeline and procedures of CRISPR/Cas9-based screening; B: Respective sgRNAs scored in negative selection CRISPR/Cas9 screening; C: Relative mRNA levels of top six sgRNAs detected by qPCR; D: KEGG pathway analysis of sgRNAs in negative selection CRISPR/Cas9 screening; E: GSEA of sgRNAs in the PI3K/AKT pathway.

To further explore the role of CBX8 in PC tumorigenesis, BXPC-3 cells were transfected with CBX8 knockdown lentiviral vectors (sh-CBX8) or empty vectors (sh-NC). A PC mouse model was established by subcutaneous injection of two groups of cells into the right flank of nude mice. The tumor size of the sh-CBX8 group was significantly smaller than that in the control group (Figure 3F). The kinetics of tumor growth of each group are shown in Figure 3H. A significant difference was found in the tumor weight between the two groups (Figure 3I).

For further investigation, immunohistochemistry (IHC) for Ki67 was performed and indicated that CBX8 knockdown could restrain the proliferative ability of PC cells (Figure 3J).



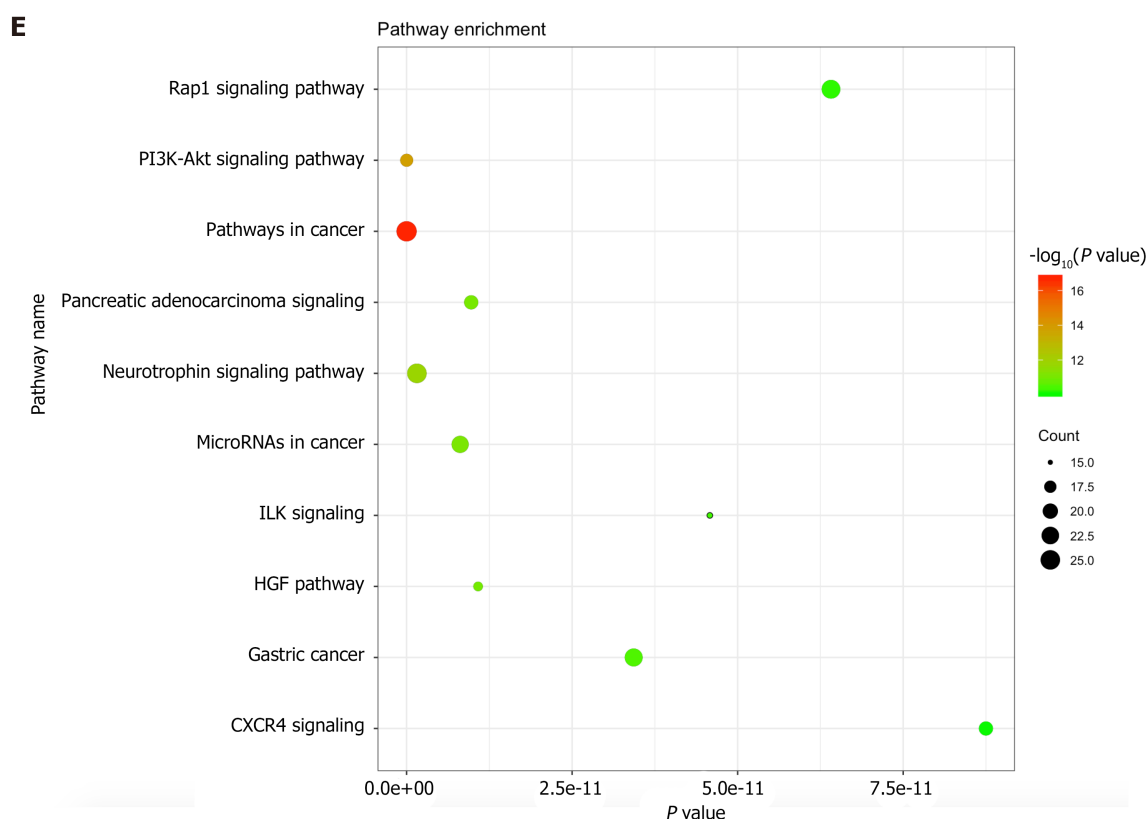


Figure 2 Higher CBX8 expression induces worse clinical outcome in pancreatic cancer tissue. A: The mRNA expression of *CBX8* in 116 paired pancreatic cancer (PC) and normal tissues measured by quantitative reverse transcription-PCR; B: Kaplan–Meier survival of patients with high and low *CBX8* mRNA level; C: Protein expression of *CBX8* in 20 paired PC and normal tissues measured by Western blot; D: Heatmap indicating genes positively related with *CBX8*; E: KEGG pathway analysis of genes in positive relation with *CBX8*. ^b $P < 0.01$.

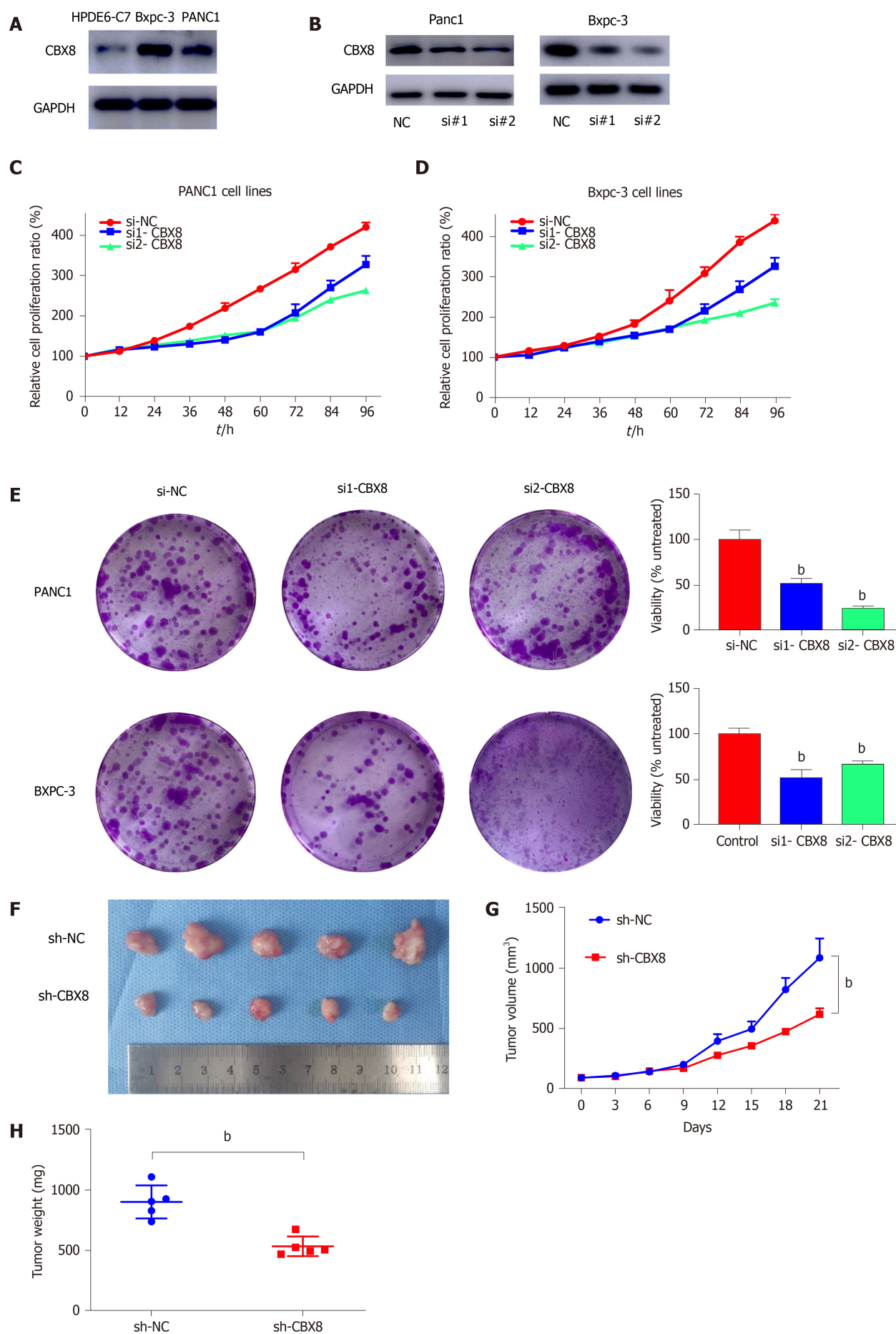
HIF-1 α modulates CBX8 expression transcriptionally in PC cells

Hypoxia is one of the important characteristics of PC. Among these complex mechanisms, HIF-1 α is an important molecule for cells to adapt to hypoxia. We sought to investigate the regulatory mechanism of HIF and *CBX8*.

By using active chromatin markers in the University of California Santa Cruz Genome Browser (<http://genome.ucsc.edu/>), we identified the proximal *CBX8* promoter and found the potential hypoxia-responsive elements (HREs) between -1100 and -875 bp before the transcriptional start site (TSS) (Figure 4A). Although HRE sequences are widely distributed in all genes, < 1% show hypoxia-dependent binding of HIFs; therefore, we evaluated the function of these HREs. We transfected the wild-type and mutant *CBX8* promoter into 293T cells and exposed them to normoxia or hypoxia. In mutant *CBX8* promoter, we deleted the -875 bp site. This demonstrated that the HRE of *CBX8* was responsive to hypoxia (Figure 4B). We knocked down HIF-1 α (Figure 4C) and detected *CBX8* mRNA level under normoxia or hypoxia, which indirectly proved the transcriptional regulation of *CBX8* by HIF-1 α (Figure 4D). Consistently, ChIP and qPCR demonstrated that HIF-1 α bound to *CBX8* promoter under hypoxia (Figure 4E). These data revealed that *CBX8* was a transcriptional target of HIF-1 α under hypoxia. Knockdown of HIF-1 α with two different siRNAs prevented accumulation of HIF-1 α protein under hypoxia (Figure 4F), and the protein expression of *CBX8* under hypoxia was not significantly different from that under normoxia (Figure 4G).

CBX8 promotes PC cell proliferation by targeting IRS1/PI3K pathway

To explore the specific mechanism of the regulation of *CBX8* on PC cell proliferation, we analyzed the transcriptome of wild-type and *CBX8* knockdown PC cells. The analysis revealed 312 differently expressed genes (DEGs) between the two groups and the heatmap showed the top 30 genes (Figure 5A and C). KEGG analysis showed that the DEGs were significantly enriched in the PI3K/AKT, Rap1, and neurotrophin signaling pathways (Figure 5B and C). To investigate the target genes regulated by *CBX8*, ChIP-seq was performed and we found 62 135 peaks compared to input signals. The pie chart indicates the *CBX8*-binding distribution (Figure 5D). Overlapping genes



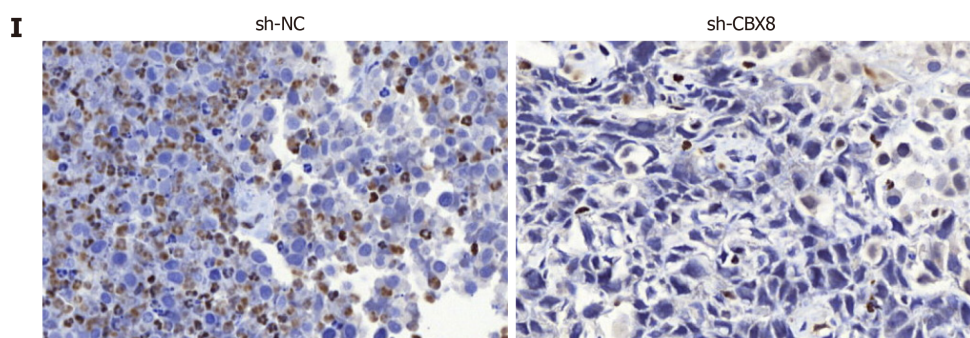


Figure 3 CBX8 is required for efficient proliferation of pancreatic cancer cells *in vitro* and *in vivo*. A: Protein expression of CBX8 in pancreatic cancer (PC) cells and normal pancreatic cells; B: Western blot analysis of CBX8 knockdown efficiency in PC cells; C: Viability of PANC1 cells after transfection with CBX8 knockdown and control plasmids; D: Viability of BXPc-3 cells after transfection with CBX8 knockdown and control plasmids; E: Colony formation assay for evaluation of the effect of CBX8 on PC cell proliferation; F: Images of tumors harvested from each group; G: Tumor growth curve drawn based on the tumor size measured each week; H: Weight of tumors in each group; I: Representative images of immunohistochemistry staining for Ki67 in xenograft tumors. ^b*P* < 0.01.

between CBX8 knockdown and the ChIP-seq data were investigated and there were 35 downregulated genes among the CBX8 target genes. IRS1, which regulates PI3K/AKT pathway activation, was in the set of target genes. We performed ATAC-seq and integrated the multiple tracks in the IGV diagram, where CBX8 and H3K27ac were found to co-occupy the *IRS1* enhancer region (Figure 5E). Upregulation of CBX8 increased the expression of IRS1 (Figure 5F), whereas CBX8 silencing led to a significant downregulation of IRS1 in PC cells (Figure 5G).

We measured CBX8 and IRS1 expression levels in PC tissues, which showed that the endogenous IRS1 level was positively correlated with CBX8 in PC tissues (Figure 5H). We conducted a luciferase reporter assay in 293T cells. The luciferase activity of *IRS1* promoter was enhanced by overexpression of CBX8 but reduced by CBX8 silencing (Figure 5I). Combined ChIP and qPCR analysis revealed that CBX8 bound to regions 2–3 in the *IRS1* promoter (Figure 5J), which were included in the -721/235 fragment. The expression of IRS1 in BXPc-3 cells was downregulated after CBX8 knockdown and upregulated after CBX8 overexpression. The phosphorylation levels of AKT and mammalian target of rapamycin (mTOR) in CBX8-overexpressing BXPc-3 cells were increased, while CBX8 knockdown suppressed the phosphorylation of AKT and mTOR (Figure 5K).

Cbx8* promotes the growth and proliferation of pancreatic cancer cells through *IRS1* *in vivo

After identifying IRS1 as the possible target of CBX8, we explored whether CBX8 promoted the proliferation of PC cells through IRS1. CCK-8 and colony formation assays were performed to assess the effects of CBX8 and IRS1 on PC cell proliferation, which indicated that CBX8 knockdown reduced the proliferation of PC cells, while IRS1 overexpression inhibited the facilitating effect of CBX8 on PC cell proliferation (Figure 6A and B). IRS1 overexpression and CBX8 knockdown plasmids were cotransfected into PC cells. IRS1 overexpression partly rescued the anti-tumorigenicity of CBX8 knockdown in PC cells (Figure 6C–E). IHC for Ki67 indicated that CBX8 knockdown decreased the proliferation of PC cells, but this was reversed partly by IRS1 overexpression (Figure 6F). In conclusion, our results showed that the CBX8/IRS1 axis regulated PC cell proliferation and HIF-1 α promoted the expression of CBX8 in PC cells under hypoxia (Figure 6G).

DISCUSSION

In recent years, CRISPR screening has made great progress in cancer research. Many studies have used CRISPR systems to screen key genes that mediate tumor drug resistance or immune escape[8–10]. In our study, we used the CRISPR screening system to screen genes related to tumor growth. We screened many growth-related genes, among which *CBX8* was associated with a poor clinical prognosis of PC, and *CBX8* deletion can slow down the proliferation of PC cells. Our data showed that knockdown of CBX8 decreased PC cell proliferation, as demonstrated by weakened colony formation *in vitro* and in mouse xenografts. The role of CBX8 in PC has not

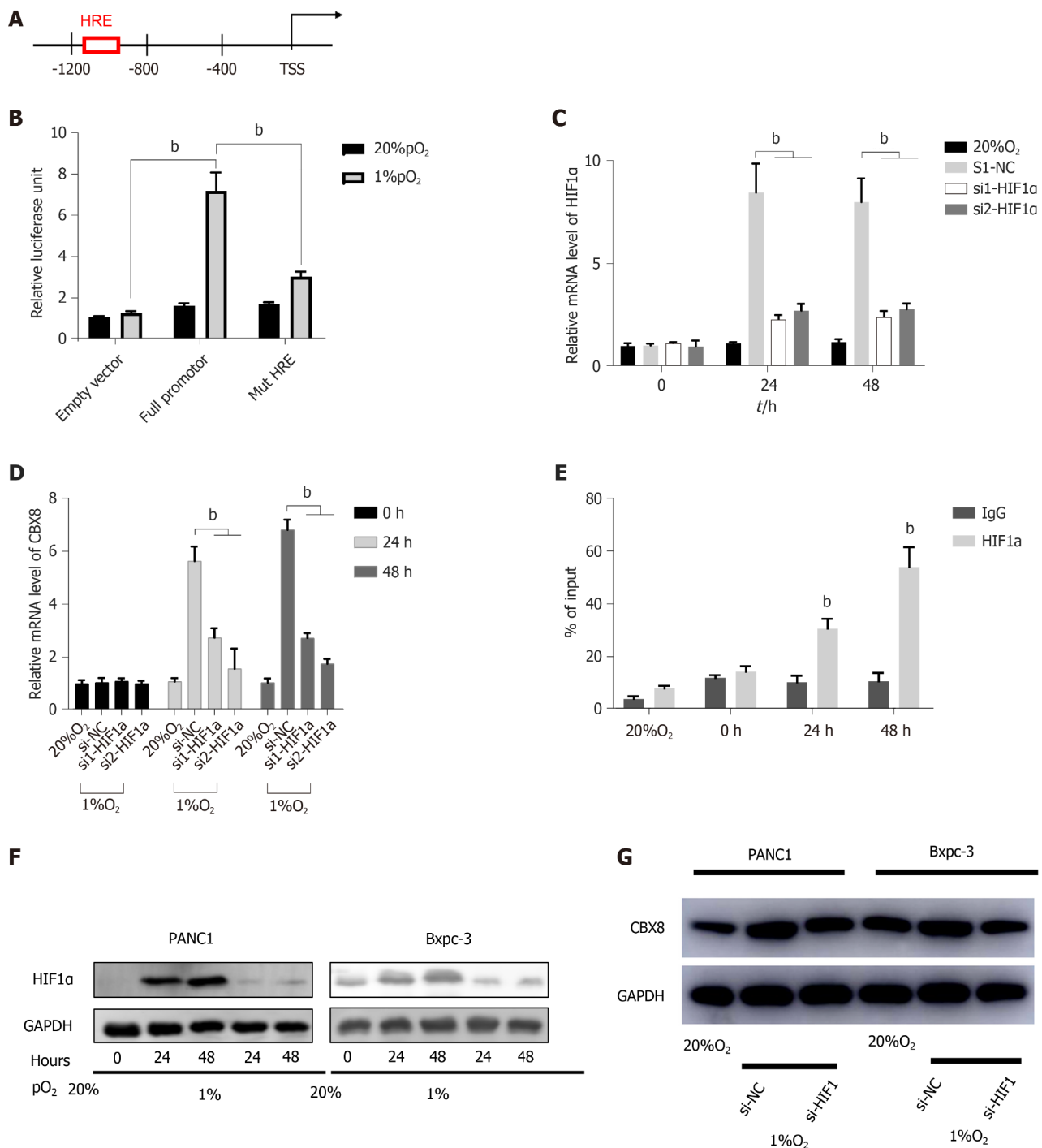
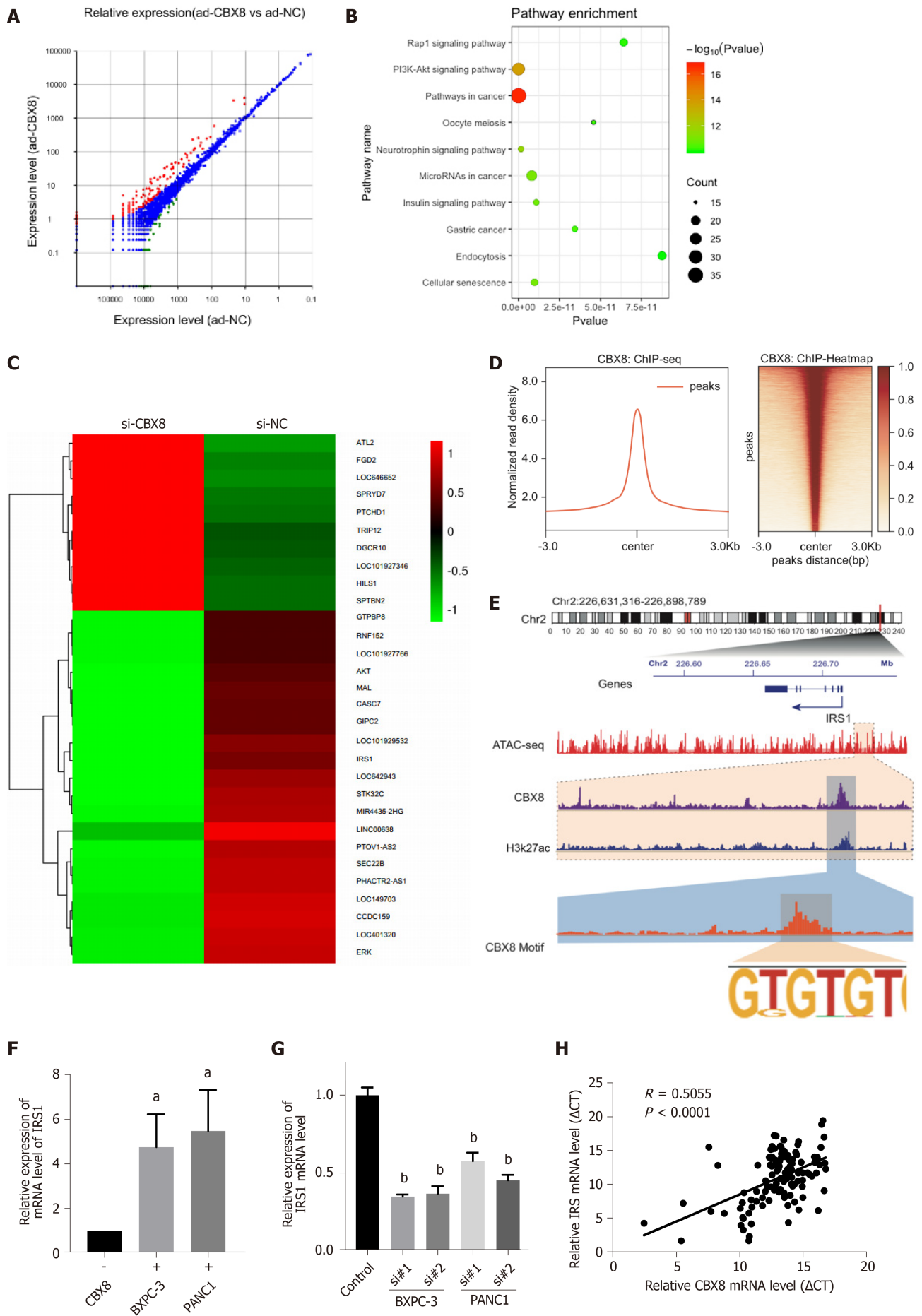


Figure 4 Hypoxia-inducible factor-1 α modulates CBX8 expression transcriptionally in pancreatic cancer cells. A: Human *CBX8* promoter shown with HRE; B: 293T cells transfected with full HRE promoter and mutant HRE were cultured under normoxia or hypoxia for 48 h, and firefly luciferase activity determined relative to control Renilla luciferase; C: Quantitative reverse transcription-polymerase chain reaction for detecting hypoxia-inducible factor-1 α (HIF-1 α) knockdown efficiency under hypoxia; D: Expression level of *CBX8* mRNA was evaluated and normalized to control group; E: Chromatin immunoprecipitation-qPCR analysis was used to determine the binding affinity of HIF-1 α to *CBX8* promoter regions; F and G: Expression levels of HIF-1 α and CBX8 protein were evaluated and normalized to control group. ^bP < 0.01.

been studied before, and it may become a new therapeutic target.

CBX8 is associated with the stemness and chemosensitivity of many types of cancer. It has been reported that CBX8 upregulates *LGR5* expression in a noncanonical manner by interacting with KMT2b and Pol II, leading to increased cancer stemness and decreased chemosensitivity in CC[5]. CBX8 also promotes the proliferation of HCC cells through YBX1-mediated cell cycle progression, and high CBX8 expression is related to a poor prognosis of HCC patients[11]. A recent study showed that CBX8 recruited KMT2b to the *LGR5* promoter and modulated H3K27me3 in the promoter of bone morphogenetic protein (BMP)4, resulting in increased *BMP4* transcription and



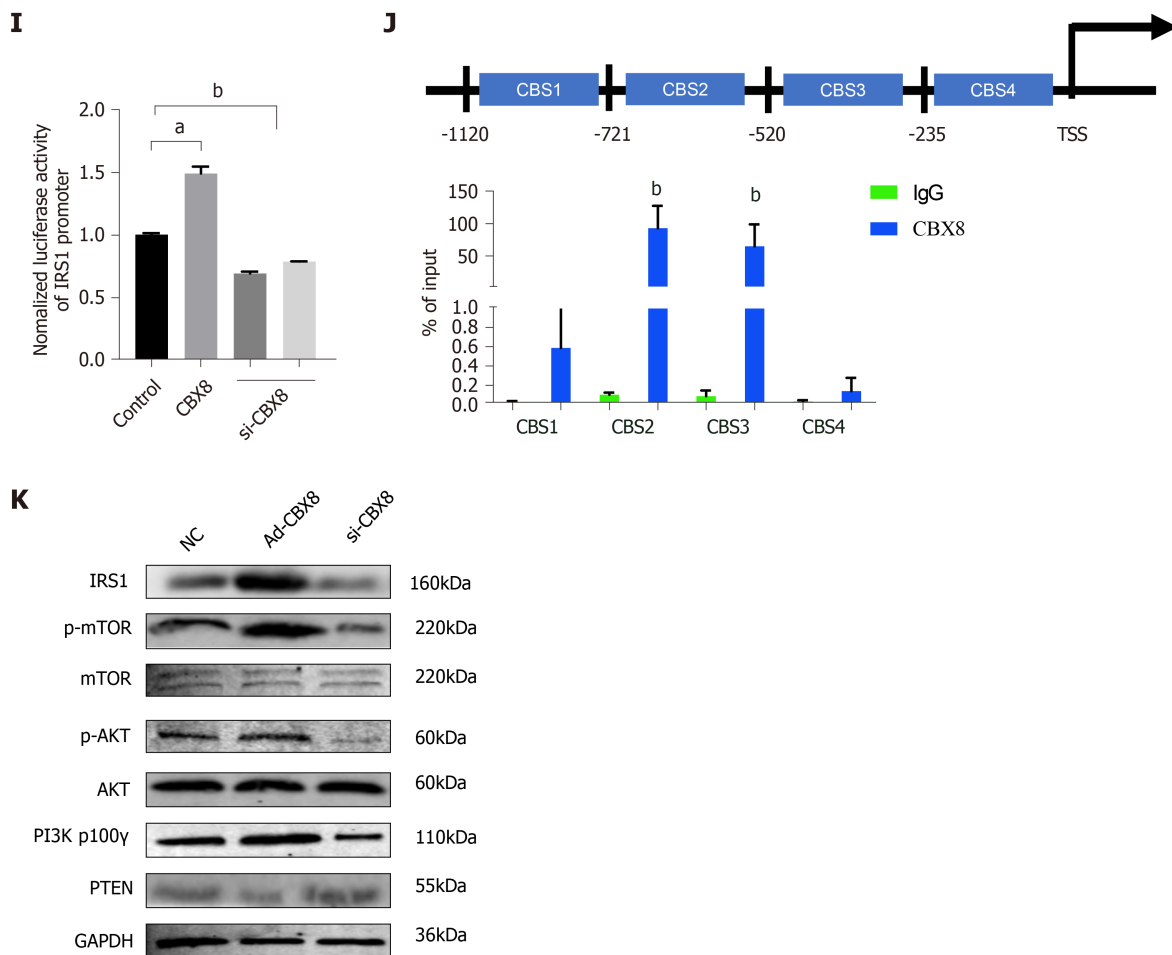


Figure 5 CBX8 promotes pancreatic cancer cell proliferation by targeting IRS1/PI3K pathway. A: Differently expressed genes (DEGs) after CBX8 knockdown; B: KEGG pathway analysis of DEGs; C: Heatmap indicating the top 30 DEGs; D: Chromatin immunoprecipitation (ChIP)-Seq summary plot of CBX8-binding intensities across CBX8 peaks in BXPC-3 cells; E: Multiple tracks in the IGV diagram exhibited the co-occupancy of CBX8 and H3K27ac in the IRS1 enhancer region; F: Expression level of *IRS1* mRNA was evaluated and normalized to the control group; G: Expression level of *IRS1* mRNA was evaluated and normalized to untransduced controls; H: Expression of CBX8 and IRS1 exhibited a positive correlation in PC tissues; I: Luciferase reporter assay was performed to confirm the binding of CBX8 to the promoter region of *IRS1* in 293T cells; J: ChIP-qPCR analysis was used to determine the binding affinity of CBX8 to *IRS1* promoter regions; K: Expression of PTEN and PI3K γ and the phosphorylation levels of IRS1, AKT, and mTOR in PC cells were assessed by Western blot. ^a*P* < 0.05, ^b*P* < 0.01.

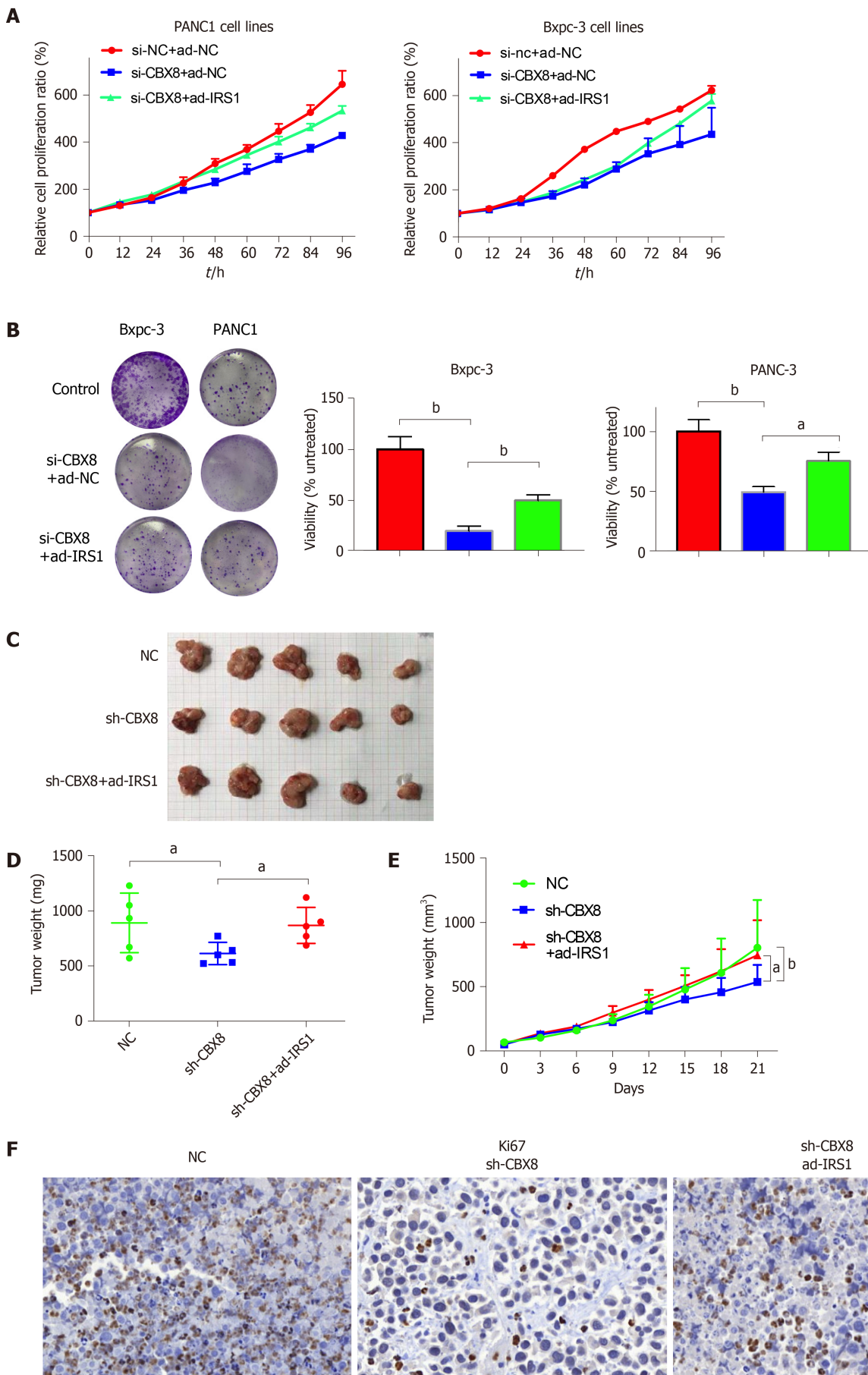
subsequent activation of Smads and mitogen-activated protein kinases. In our study, the level of CBX8 in PC tissues was higher than that in adjacent normal tissues. The CRISPR/Cas9 screening also indicated that CBX8 knockout might decrease the proliferation of PC cells.

Hypoxia is a significant feature of PC. Due to the rapid growth of tumor, the blood vessels in PC often show immature microvascular lumina. This results in hypoxic features in PC tissues. In our previous studies, we found that hypoxia mediated the invasion, metastasis, and angiogenesis of PC by promoting the secretion of exosomes [4,12]. In the present study, we found that hypoxia-upregulated HIF-1 α bound to CBX8 promoter region and regulated CBX8 expression at the transcriptional level.

The IRS family is composed of four proteins (IRS1–IRS4), which were initially considered as typical cytosolic adaptor proteins. They are involved in IR and insulin-like growth factor I receptor signal transduction [13,14]. Post-translational modification of IRS1 can activate the mTORC1 signaling pathway through chronic elevation of multiple serine phosphorylation sites [15].

In our study, CBX8 modulated H3K27me₃ in the promoter of *IRS1*, resulting in increased *IRS1* transcription and subsequent activation of PI3K and AKT. At the same time, *in vitro* colony formation assay and mouse xenograft model confirmed that CBX8 promoted the growth of PC cells through IRS1.

Through CRISPR screening, we identified a group of genes related to the growth of PC. Combined analysis of clinical samples of patients with PC demonstrated that CBX8 was higher in PC tumor tissue and high expression of CBX8 predicted a poor clinical outcome. HIF-1 α regulated expression of CBX8 transcriptionally under



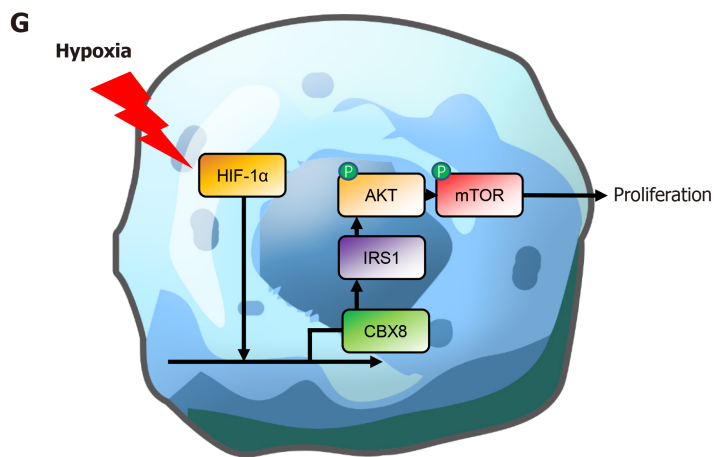


Figure 6 CBX8 promotes the growth and proliferation of pancreatic cancer cells through IRS1 *in vivo*. A: Viability of BXPc-3 and PANC1 cells after transfection with CBX8 knockdown and/or IRS1 plasmid; B: Colony formation assay was performed to evaluate the effect of CBX8 on PC cell proliferation; C: Images of tumors harvested from each group; D: Weight of tumors in each group; E: Tumor growth curve drawn based on the tumor size measured each week; F: Representative images of immunohistochemistry staining for Ki67 in xenograft tumors; G: Schematic model illustrating that HIF-1 α -mediated CBX8 transcription promotes pancreatic cancer progression *via* IRS1/AKT axis. ^a $P < 0.05$, ^b $P < 0.01$.

hypoxia and CBX8 induced PC cell proliferation by targeting IRS1, which activated the PI3K/AKT pathway. These studies revealed the mechanism of the promotion effect of CBX8 on the development of PC, and provided potential therapeutic targets.

CONCLUSION

Our results suggest that CBX8 could function as an oncogenic factor in PC progression. High CBX8 expression is correlated with poor clinical outcomes of PDAC patients from two independent cohorts. We also showed that CBX8 is a key gene that is regulated by HIF-1 α , and can activate the IRS1/AKT pathway. The above findings suggest that targeting CBX8 may be a promising therapeutic strategy for PC.

ARTICLE HIGHLIGHTS

Research background

Pancreatic cancer (PC) is one of the most lethal cancers worldwide. It has become the second most fatal cancer in the United States. Chromobox (CBX)8 promotes tumor growth and metastasis in other cancers. However, whether CBX8 is involved in the proliferation of PC cells remains unknown.

Research motivation

Many studies have shown that the prognosis of patients with PC remains poor after complete surgical resection. Therefore, it is important to study the occurrence and development of PC and the corresponding targeted therapy. We hope to provide a novel therapeutic target for patients with PC.

Research objectives

The present study aimed to investigate the function of the CBX8/IRS1/AKT axis in PC.

Research methods

Genome-wide CRISPR-Cas9 screening was performed to select genes that could facilitate PC cell proliferation. A total of 244 candidate genes were identified as being responsible for proliferation of PC cells using deep single guide RNA sequencing. Quantitative reverse transcription-polymerase chain reaction was used to detect the expression of CBX8 in PC tissues and cells. The regulatory roles of CBX8 in cell proliferation, migration, and invasion were verified by CCK-8 and Transwell assays.

Research results

CBX8 was upregulated in pancreatic tumor tissues and shown to drive PC cell proliferation. Higher expression of CBX8 was correlated with worse outcomes of PC patients from two independent cohorts with a total of 116 cases. CBX8 also served as a promising therapeutic target for a PC xenograft model. We demonstrated that HIF-1 α induced CBX8 transcription by binding to the promoter of CBX8. CBX8 efficiently activated the PI3K/AKT signaling pathway by upregulating insulin receptor substrate (IRS)1.

Research conclusions

CBX8 promotes PC cell progression by activating the IRS1/AKT pathway.

Research perspectives

CBX8 could promote PC progression, which might provide a potential treatment strategy for this malignancy.

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REFERENCES

- 1 **Balachandran VP**, Beatty GL, Dougan SK. Broadening the Impact of Immunotherapy to Pancreatic Cancer: Challenges and Opportunities. *Gastroenterology* 2019; **156**: 2056-2072 [PMID: [30660727](#) DOI: [10.1053/j.gastro.2018.12.038](#)]
- 2 **Choudhry H**, Harris AL. Advances in Hypoxia-Inducible Factor Biology. *Cell Metab* 2018; **27**: 281-298 [PMID: [29129785](#) DOI: [10.1016/j.cmet.2017.10.005](#)]
- 3 **Wang X**, Luo G, Zhang K, Cao J, Huang C, Jiang T, Liu B, Su L, Qiu Z. Hypoxic Tumor-Derived Exosomal miR-301a Mediates M2 Macrophage Polarization via PTEN/PI3K γ to Promote Pancreatic Cancer Metastasis. *Cancer Res* 2018; **78**: 4586-4598 [PMID: [29880482](#) DOI: [10.1158/0008-5472.CAN-17-3841](#)]
- 4 **Guo Z**, Wang X, Yang Y, Chen W, Zhang K, Teng B, Huang C, Zhao Q, Qiu Z. Hypoxic Tumor-Derived Exosomal Long Noncoding RNA UCA1 Promotes Angiogenesis via miR-96-5p/AMOTL2 in Pancreatic Cancer. *Mol Ther Nucleic Acids* 2020; **22**: 179-195 [PMID: [32942233](#) DOI: [10.1016/j.omtn.2020.08.021](#)]
- 5 **Zhang Y**, Kang M, Zhang B, Meng F, Song J, Kaneko H, Shimamoto F, Tang B. m⁶A modification-mediated CBX8 induction regulates stemness and chemosensitivity of colon cancer via upregulation of LGR5. *Mol Cancer* 2019; **18**: 185 [PMID: [31849331](#) DOI: [10.1186/s12943-019-1116-x](#)]
- 6 **Zhang CZ**, Chen SL, Wang CH, He YF, Yang X, Xie D, Yun JP. CBX8 Exhibits Oncogenic Activity via AKT/ β -Catenin Activation in Hepatocellular Carcinoma. *Cancer Res* 2018; **78**: 51-63 [PMID: [29066512](#) DOI: [10.1158/0008-5472.CAN-17-0700](#)]
- 7 **Chung CY**, Sun Z, Mullokandov G, Bosch A, Qadeer ZA, Cihan E, Rapp Z, Parsons R, Aguirre-Ghiso JA, Farias EF, Brown BD, Gaspar-Maia A, Bernstein E. Cbx8 Acts Non-canonically with Wdr5 to Promote Mammary Tumorigenesis. *Cell Rep* 2016; **16**: 472-486 [PMID: [27346354](#) DOI: [10.1016/j.celrep.2016.06.002](#)]
- 8 **Huang K**, Liu X, Li Y, Wang Q, Zhou J, Wang Y, Dong F, Yang C, Sun Z, Fang C, Liu C, Tan Y, Wu X, Jiang T, Kang C. Genome-Wide CRISPR-Cas9 Screening Identifies NF- κ B/E2F6 Responsible for EGFRvIII-Associated Temozolomide Resistance in Glioblastoma. *Adv Sci (Weinh)* 2019; **6**: 1900782 [PMID: [31508283](#) DOI: [10.1002/advs.201900782](#)]
- 9 **Li Z**, Zhou X, Wei M, Gao X, Zhao L, Shi R, Sun W, Duan Y, Yang G, Yuan L. In Vitro and in Vivo RNA Inhibition by CD9-HuR Functionalized Exosomes Encapsulated with miRNA or CRISPR/dCas9. *Nano Lett* 2019; **19**: 19-28 [PMID: [30517011](#) DOI: [10.1021/acs.nanolett.8b02689](#)]
- 10 **Dufva O**, Koski J, Maliniemi P, Ianevski A, Klievink J, Leitner J, Pölönen P, Hohtari H, Saeed K, Hannunen T, Ellonen P, Steinberger P, Kankainen M, Aittokallio T, Keränen MAI, Korhonen M, Mustjoki S. Integrated drug profiling and CRISPR screening identify essential pathways for CAR T-cell cytotoxicity. *Blood* 2020; **135**: 597-609 [PMID: [31830245](#) DOI: [10.1182/blood.2019002121](#)]
- 11 **Xiao L**, Zhou Z, Li W, Peng J, Sun Q, Zhu H, Song Y, Hou JL, Sun J, Cao HC, Zhongyi D, Wu D, Liu L. Chromobox homolog 8 (CBX8) Interacts with Y-Box binding protein 1 (YBX1) to promote cellular proliferation in hepatocellular carcinoma cells. *Aging (Albany NY)* 2019; **11**: 7123-7149 [PMID: [31495785](#) DOI: [10.18632/aging.102241](#)]
- 12 **Keshavarzi F**, Golsheh S. IRS1-rs10498210 G/A and CCR5-59029 A/G polymorphisms in patients with type 2 diabetes in Kurdistan. *Mol Genet Genomic Med* 2019; **7**: e631 [PMID: [30884193](#) DOI: [10.1002/mgg2.1006](#)]

- 10.1002/mgg3.631]
- 13 **Esposito DL**, Aru F, Lattanzio R, Morgano A, Abbondanza M, Malekzadeh R, Bishehsari F, Valanzano R, Russo A, Piantelli M, Moschetta A, Lotti LV, Mariani-Costantini R. The insulin receptor substrate 1 (IRS1) in intestinal epithelial differentiation and in colorectal cancer. *PLoS One* 2012; **7**: e36190 [PMID: [22558377](#) DOI: [10.1371/journal.pone.0036190](#)]
 - 14 **Ardestani A**, Maedler K. mTORC1 and IRS1: Another Deadly Kiss. *Trends Endocrinol Metab* 2018; **29**: 737-739 [PMID: [30082207](#) DOI: [10.1016/j.tem.2018.07.003](#)]



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