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Expression of HMGB2 indicates worse survival of patients and is required for the maintenance of Warburg effect in pancreatic cancer

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Abstract

High mobility group proteins (HMGs) are the second most abundant chromatin proteins and exert global genomic functions in the establishment of active or inactive chromatin domains. Through interaction with nucleosomes, transcription factors, nucleosome-remodeling machines and histones, the HMGs family proteins contribute to the fine tuning of transcription in response to rapid environmental changes. Mammalian high mobility group Bs (HMGBs) are characterized by two tandem HMG box domains followed by a long acidic tail. Recent studies demonstrated that high expression of HMGBs has been found in many cancers, such as prostate, kidney, ovarian, and gastric cancers. However, their roles in pancreatic cancer have seldom been reported. In this study, we assessed the diagnostic and prognostic values of HMGBs proteins, including HMGB1, HMGB2, and HMGB3, in pancreatic cancer from the Cancer Genome Atlas (TCGA) dataset. Our results demonstrated that HMGB2 predicted poor prognosis in pancreatic cancer. *In vitro* studies demonstrated that silencing HMGB2 inhibited cell proliferation and viability. Mechanistically, our results demonstrated that silencing HMGB2 decreased hypoxia inducible factor 1α (HIF1α) protein level and inhibited HIF1α-mediated glycolysis process. Further analysis indicated that HIF1α-targeted glycolytic genes, including *GLUT1*, *HK2*, and *LDHA*, are all prognostic factors and positively correlated with HMGB2 expression. Taken together, we discovered new prognostic and predictive markers for pancreatic cancer, and shed light on the novel function of HMGB2 in glycolytic control in cancer.

Key words: pancreatic cancer, HMGB2, Warburg effect

Introduction

Pancreatic cancer is an aggressive disease and is the forth lethal disease related to cancers in developed countries. Despite the dramatic progress in treatment and in understanding the molecular mechanisms of carcinogenesis, the prognosis of pancreatic cancer remains poor. The median survival after diagnosis is around 3–6 months, and the 5-year survival rate is ~5% [1–4]. Pancreatic ductal adenocarcinoma (PDAC) represents more than 90% of newly

emerging pancreatic cases, and its prognosis is worse than other cancers with mortality rate reaching as high as 99% [5]. Therefore, there is an urgent need for understanding the molecular mechanisms of the oncogenic and metastatic process of pancreatic cancer. Moreover, the development of efficient strategies and the search of novel predictive markers become more pressing than ever.

The genetic landscape of pancreatic cancer shows near ubiquitous activating mutations of KRAS, and recurrent inactivating mutations of CDKN2A, SMAD4, and TP53. The genetic mutations of pancreatic cancer endow the cancer with malignant properties such as uncontrolled proliferation and metastasis [6]. It is well acknowledged that proliferating human cells require more energy and material supply to sustain the proliferative process. However, rapidly proliferative solid tumor cells lack supplies of oxygen and nutrients due to limited oxygen supply caused by severely hypoxic conditions. To survive under such hypoxic conditions, tumor cells must shift their metabolic pattern to adapt to the hostile microenvironment, and this phenomenon is known as metabolic reprogramming [7]. Altered tumor metabolism is now a generally accepted hallmark of cancer, and has received more attention than ever before [8]. The initial recognition that cancer cells exhibit atypical metabolic characteristics can be traced to the pioneering work of Otto Warburg who discovered that rapidly proliferating cancer cells metabolized glucose into lactate under aerobic conditions [9]. Though this process is far less efficient in terms of adenosine triphosphate production, it provides cancer cells with building blocks for macromolecule synthesis, necessary redox conditions and energies required. This phenomenon is called Warburg effect [9–11]. Hypoxia inducible factor 1α (HIF1α) and c-myc are considered to be important regulators of glucose metabolism reprogramming [12]. To fuel uncontrolled proliferation of cancer cells, HIF1α or c-myc reprograms cancer cell metabolism in a way that is different from normal cells. HIF1α- or c-myc-induced metabolic signatures are characterized by enhanced glucose and glutamine uptake, increased lactate production, and altered amino acid or lipid metabolism [13]. Presumably, the alternative therapeutic strategy for the treatment of cancer is the combined targeting of multiple HIF1α- or c-myc-mediated pathways that are involved in regulating cell proliferation, metabolism, and oncogenic stress. HIF1α and c-myc are transcription factors, which exert their function by initiating a series of transcriptional process through regulating chromatin structures. HIF1α and c-myc exert their function through association with a series of epigenetic regulators, including DNA methyltransferase, DNA hydroxylases, histone acetyltransferases, histone deacetylases, histone methyltransferases, and histone demethylases [14].

Besides histones, high mobility group proteins (HMGs) are the second most abundant proteins and exert global genomic functions in establishing active or inactive chromatin domains. Through interaction with nucleosomes, transcription factors, nucleosome-remodeling complexes, and histone H1, HMGs contribute to the fine tuning of transcription in response to rapid environmental changes [15,16]. Among the HMGs family, high mobility group Bs (HMGBs) have been previously reported to be related to the onset and progression of cancers of different origins, such as prostate cancer, bladder cancer, hepatocellular carcinoma, gastric cancer, and lung cancer [17–21]. Furthermore, upregulation of HMGB members is associated with many hallmarks of cancers, including uncontrolled replicative capacity, evasion of apoptosis, and tissue invasion and metastasis, and these results suggest that HMGB is a new potential therapeutic target for the treatment of human malignancies [22]. Several studies have assessed the diagnostic and prognostic values of HMGB proteins in cancers, but results remain controversial. Thus in this study, we performed a thorough analysis of the expression of HMGB1-3 from the Cancer Genome Altlas (TCGA) dataset. It was found that HMGB2 expression mainly affects the survival rates of pancreatic cancer. We, therefore, used *in vitro* function studies to examine the role of HMGB2 in pancreatic cancer and its effector pathways to better understand the mechanism by which HMGB2 contributes to the development and progression of pancreatic cancer.

Materials and Methods

Cell culture

The human pancreatic cancer cell lines PANC-1 and MIA PaCa-2 were obtained from the American Type Culture Collection (ATCC, Manassas, USA) and cultured according to the standard ATCC protocols. In brief, PANC-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), containing fetal bovine serum (FBS) in a final concentration of 10%. MIA PaCa-2 cells were cultured in DMEM, with FBS in a concentration of 10% and horse serum in a concentration of 2.5%.

RNA isolation and quantitative real-time PCR

Total RNA was prepared using Trizol reagent (Invitrogen, Carlsbad, USA). To obtain cDNA, PrimeScript RT reagent kit (TaKaRa, Dalian, China) was used for reverse transcription. Expression levels of candidate genes and *β-actin* were determined by quantitative real-time PCR using an ABI 7900HT Real-Time PCR system (Applied Biosystems, Foster City, USA). All reactions were run in triplicate. Sequences of primers are listed in **Table 1**.

Protein extraction and western blot analysis

Cells were washed twice with ice-cold PBS and lysed in RIPA buffer for 10 min. Cell debris was removed by centrifugation at 12,000 *g* for 20 min at 4°C. Total protein lysates (20 μg) were subject to electrophoresis in denaturing 10% sodium dodecyl sulfate-polyacrylamide gel, and then transferred to a membrane for subsequent blotting incubation with antibodies against HMGB2, β-actin, HIF1α, HK2, Glut1, and LDHA that were purchased from Proteintech (Rosemont, USA) and antibodies against AKT1, pAKT1, mTOR, and p-mTOR that were obtained from Abcam (Cambridge, UK).

Lentivirus production and stable cell line selection

In order to generate shRNA expression constructs against *HMGB2*, pLKO.1 TRC cloning vector (Addgene plasmid: 10878) was employed [23]. Targets against *HMGB2* (21 bp) were 5′-GCT

Table 1. Sequence of primers used in this study

Gene	Primer sequence (5′→3′)
HMGB2 (F)	GGACCCCAATGCTCCTAAAAGGCC
HMGB2 (R)	TGCCCTTGGCACGATATGCAGCA
Glut1 (F)	TGTCGTGTCGCTGTTTGTGGTGGA
Glut1 (R)	TGAAGAACAGAACCAGGAGCACAG
HK2 (F)	TGATGTGGCTGTGGATGAGCT
HK2 (R)	GCCAGGCAGTCACTCTCAATCTG
LDHA (F)	AGGCTGGGAGTTCACCCATTAAGC
LDHA (R)	GAGTCCAATAGCCCAGGATGTG
β-actin (F)	CTACGTCGCCCTGGACTTCGAGC
β-actin (R)	GATGGAGCCGCCGATCCACACGG

F, forward; R, reverse.

CAATACTAGCTTCAGTAT-3′ and 5′-CCATCTGCCTTCTTCCT GTTT-3′, respectively. Lentiviral particles were produce by co-transfection of pLKO.1-shHMGB2 constructs with psPAX2 and pMD2.G into HEK-293T cells in a ratio of 4:3:1. Cell lines were obtained by infection of PANC-1 and MIA PaCa-2 cells with lentiviral particles followed by puromycin selection.

CCK-8 proliferation assay

Cell proliferation was determined by CCK-8 assays using CCK-8 reagents (Dojindo, Tokyo, Japan) and performed according to the manufacturer's protocol. In brief, 2×10^3 cells/well were seeded in 96-well culture plates and incubated for 4 days at 37°C with 5% CO_2 in a humidified incubator. Viable cells were quantified by adding CCK-8 solution followed by measuring the absorbance at 450 nm every 24 h.

Colony-formation assay

PANC-1 and MIA PaCa-2 cells (5×10^2) stably expressing shRNA targets against *HMGB2* and its relative control cells were seeded in six-well plates. Every 3–4 days, the medium should be replaced with fresh medium. The colonies were visible after cultivating for 10–15 days, and then the cells were fixed with 4% paraformaldehyde at room temperature for 5 min. Then the cells were stained with 1% crystal violet and incubated at room temperature for 2 h. Remove crystal violet carefully and immerse the plates in ddH_2O to rinse off crystal violet. After the plates were dried at room temperature, the number of colonies was counted.

Immunohistochemical staining

This study was approved by the Institutional Research Ethics Committee of Minhang Hospital, Fudan University, and written consents were obtained from five patients with pathologically and clinically confirmed pancreatic cancer. The surgical resected tissues were fixed in formalin and embedded in paraffin for histological analysis and immunohistochemical studies. The antibodies against HMGB2 and HIF1α were used to detect their expression according to standard procedures described previously [24]. Briefly, thin sections of 10% formalin-fixed, paraffin-embedded tissue specimens were treated with rabbit anti-human HMGB2 (14597-1-APl; Proteintech) and HIF1α (20960-1-AP; Proteintech) antibodies, followed by treatment with peroxidase-conjugated goat anti-rabbit (sc-2018; Santa Cruz Biotech, Santa Cruz, USA) secondary antibody. The percentage of positively stained cancer cells was determined by two independent pathologists and classified into two groups: negative staining (no cells were intensely stained), and positive staining (at least 10% cells were intensely stained) [25].

Measurement of oxygen consumption rate and extracellular acidification rate

Cellular mitochondrial function and glycolytic capacity were determined by measuring the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using the Seahorse XF Cell Mito stress test kit and Glycolysis stress test kit, respectively, with the Seahorse Bioscience XF96 Extracellular Flux Analyzer (Seahorse Bioscience, Norwalk, USA), according to the manufacturer's instructions [26].

Dual-luciferase assay

Hypoxia response element (HRE) promoter activity was determined by dual-luciferase assay. Cells were seeded into 96-well culture plates and transfected using Lipofectamine™ 2000 (Invitrogen). HRE-luciferase plasmid (Addgene plasmid 26731) and the *Renilla* luciferase expression vector pRL-TK (Promega, Madison, USA) were transfected into PANC-1 and MIA PaCa-2 cells. Then, the cells were assayed for both firefly and *Renilla* luciferase activities using a dual-luciferase system (Promega) according to the manufacturer's protocol.

TCGA dataset analysis

The Cancer Genome Atlas of pancreatic adenocarcinoma (TCGA-PAAD) on RNA expression (Level 3) of pancreatic cancer patients in terms of RNA-seq by Expectation-Maximization was downloaded from the Cancer Genomics Brower of the University of California, Santa Cruz (UCSC) (https://genome-cancer.ucsc.edu/). A total of 160 primary pancreatic cancer samples from patients with detailed expression data were chosen from the updated TCGA database according to the parameters mentioned. Detailed demographics of these patients, characterized by the TCGA consortium, are shown in **Table 2**.

Statistical analyses

Statistical analyses were performed by SPSS software (version 17.0; IBM Corp., Armonk, USA) using independent *t* tests (for continuous variables) and Pearson's χ^2 tests (for categorical variables). Logistic regression was used to determine the correlation between HMGB2, Glut1, HK2, and LDHA expression levels and clinicopathological characteristics in the TCGA cohorts. Statistical significance was based on two-sided *P* values of <0.05.

Table 2. Clinicopathological features and correlation of HMGB2 expression in PDAC in the TCGA cohorts

Characteristics	No.	HMGB2-low 80	HMGB2-high 80	*P* value
Age (year)				1
<60	50	25	25	
≥60	110	55	55	
Gender				0.8738
Female	72	35	37	
Male	88	45	43	
Tumor size (cm)				1
<4.0	91	45	46	
≥4.0	69	35	34	
Tumor differentiation				1
Well/moderate	112	56	56	
Poor	48	24	24	
Pathological N				0.4877
N0	47	26	21	
N1	113	54	59	
Pathological M				0.6202
M0	156	79	77	
M1	4	1	3	
Pathological T				0.2982
T1/T2	28	17	11	
T3/T4	132	63	69	
Stage				0.2846
I–IIA	43	25	18	
IIB–VI	117	55	62	

P values were derived with Fisher's exact test; all statistical tests are two-sided.

Results

HMGB2 predicts poor prognosis in pancreatic cancer

HMGB family members, including HMGB1, HMGB2, and HMGB3, have indispensable functions. To assess which one has indicative value in predicting prognosis of pancreatic cancer, overall survival analysis was performed using the TCGA-PAAD dataset. Results demonstrated that HMGB2 has predictive value in pancreatic cancer prognosis, while HMGB1 and HMGB3 indicated no significance in predicting overall survival (**Fig. 1**).

Silencing HMGB2 inhibits cell proliferation *in vitro*

To assess the function of HMGB2 in proliferative control in pancreatic cancer, HMGB2 expression was silenced by lentivirus-mediated transfection. Two shRNA oligos could effectively decreased HMGB2 at transcription level in PANC-1 and MIA PaCa-2 cells (**Fig. 2A**). Moreover, the efficacy was further validated by the immunoblot analysis (**Fig. 2B**). To assess the role of HMGB2 in cell viability, CCK-8 proliferation assay was performed, and our results demonstrated that inhibition of HMGB2 expression attenuated cell proliferation (**Fig. 2C**). Furthermore, colony-formation assay demonstrated that silencing HMGB2 expression resulted in the reduction of clone formation capacity in PANC-1 and MIA PaCa-2 cells (**Fig. 2D,E**). Taken together, these results validated HMGB2 as a positive regulator of cell proliferation in pancreatic cancer cells.

HMGB2 modulates AKT/mTOR/HIF1α signaling pathway

It has been demonstrated that HMGB2 can regulate AKT signaling pathway and is a modulator of pluripotency [27]; however, its role in the AKT signaling pathway in pancreatic cancer has never been reported. Thus, the activation status of AKT signaling pathway in HMGB2 silencing PANC-1 and MIA PaCa-2 cells was examined. In HMGB2-knockdown cells, activation of AKT1 was attenuated. Moreover, the downstream activation of mTOR was also inhibited accordingly. HIF1α is a well-accepted downstream target of the AKT/mTOR signaling pathway. As expected, a decrease of HIF1α protein level in HMGB2-knockdown PANC-1 and MIA PaCa-2 cells was detected (**Fig. 3A**). To further validate the correlation between HMGB2 and HIF1α, IHC staining was performed using anti-HMGB2 and anti-HIF1α antibodies in pancreatic cancer patient samples. Results demonstrated that HIF1α exhibited a higher expression in patients with high HMGB2 expression (**Fig. 3B**). These results further validated the correlation between HMGB2 and HIF1α.

HMGB2 regulates HIF1α-mediated glycolysis in pancreatic cancer cells

HIF1α is an important regulator of anabolic glycolysis and mitochondrial respiration. To further validate the physiological function of HMGB2 in HIF1α-mediated glycolysis process, *in vitro* glycolysis assay was performed using Seahorse XF24 analyzer. ECAR is an indicator of glycolytic rate. In HMGB2-knockdown PANC-1 and MIA PaCa-2 cells, a significant reduction of ECAR was observed, suggesting that HMGB2 is a positive regulator of glycolysis (**Fig. 4A**). Under hypoxia conditions, the mitochondrial glucose oxidation is inhibited, and OCR is an indicator of such oxidative conditions. As observed, in HMGB2-knockdown PANC-1 and MIA PaCa-2 cells, OCR was increased significantly (**Fig. 4B**). Taken together, these results validated HMGB2 as a positive regulator of anabolic glycolysis.

HMGB2 is a positive regulator of *HIF1α* transcription

HIF1α regulates anabolic glycolysis through transcriptional regulation of a series of glycolytic genes. These genes are characterized by HRE in their promoter region, which are recognized by HIF1α for subsequent transcription. HRE-luciferase is commonly used to reflect *HIF1α* transcription activity. To verify whether HMGB2-regulated glycolysis is the result of its influence on *HIF1α* transcription activity, luciferase reporter assay was performed to check its effect on HRE-luciferase activity. In PANC-1 and MIA PaCa-2 cells, HMGB2 was found to regulate HRE-luciferase activity in a dose-dependent manner (**Fig. 5A**). *GLUT1*, *HK2*, and *LDHA* are HIF1α-targeted genes that regulate glucose uptake and metabolism into lactate in the glycolytic process. To further confirm the function of HMGB2 in HIF1α transcription, the expressions of GLUT1, HK2, and LDHA in HMGB2-knockdown PANC-1 and MIA PaCa-2 cells were examined. As expected, the expressions of GLUT1, HK2, and LDHA decreased significantly when HMGB2 was silenced (**Fig. 5B**).

HMGB2 expression is positively correlated with GLUT1, HK2, and LDHA in pancreatic cancer patients

In vitro assays demonstrated that HMGB2 positively regulated *HIF1α* transcription and its downstream targets, including *GLUT1*, *HK2*, and *LDHA*. To further confirm the effect of HMGB2 on the expressions of GLUT1, HK2, and LDHA, analysis of the TCGA dataset was performed to examine the correlation between HMGB2 with GLUT1, HK2, and LDHA, respectively. Our results demonstrated that HMGB2 expression is positively correlated with the expressions of *GLUT1*, *HK2*, and *LDHA* as revealed by the TCGA dataset analysis (**Fig. 6**).

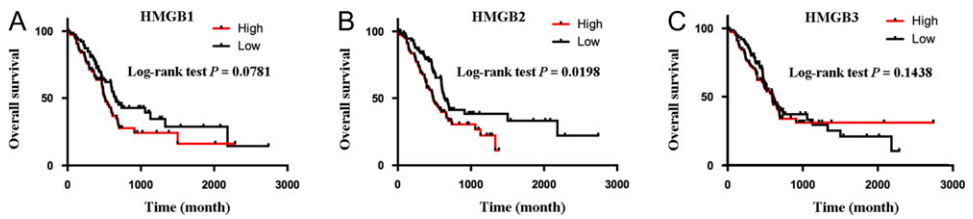

Figure 1. Application of HMGB2 in the prediction of prognosis in pancreatic cancer To assess the application of HMGBs in pancreatic cancer overall survival prediction, the expression status of HMGB1-3 was analyzed in the TCGA dataset. (A) Expression of HMGB1 displayed no contribution in overall survival prediction. (B) Higher expression level of HMGB2 indicated worse prognosis, and was a predictive marker for outcome in pancreatic cancer patients. (C) The contribution of HMGB3 to overall survival was analyzed, and the results demonstrated that HMGB3 had no significant value in pancreatic cancer overall survival prediction.

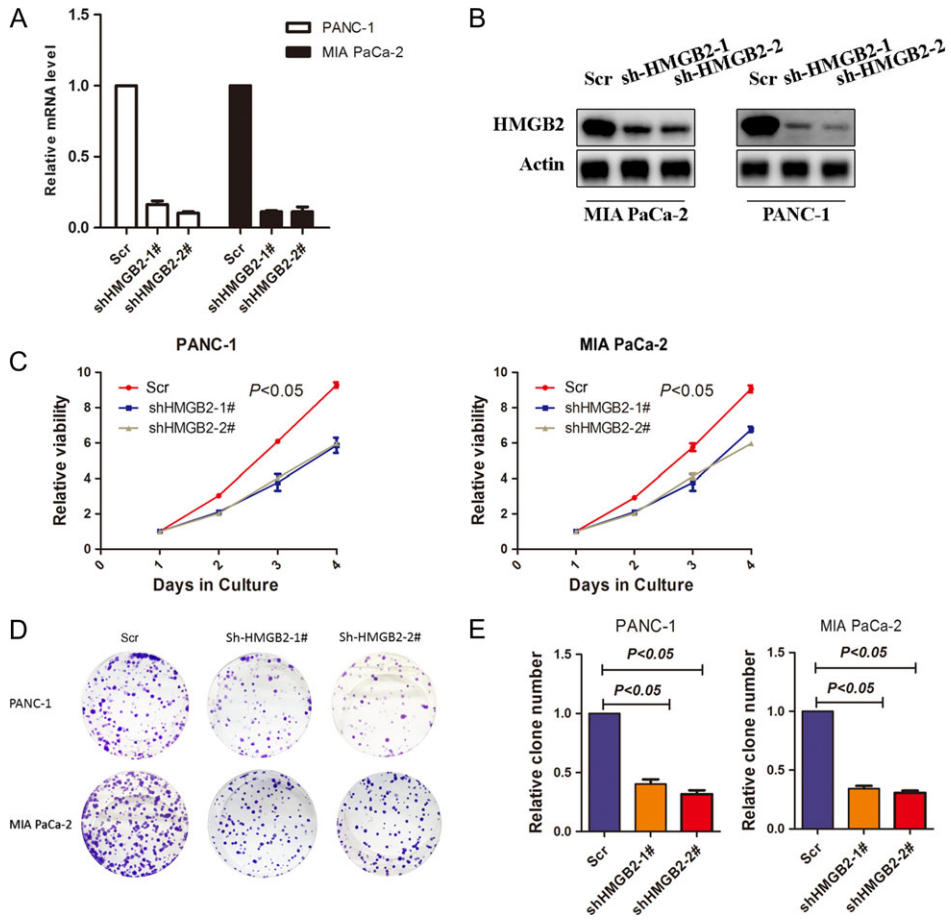

Figure 2. Silencing HMGB2 inhibits cell proliferation *in vitro* To assess the function of HMGB2 in pancreatic cancer proliferation, HMGB2 expression was silenced by shRNA in PANC-1 and MIA PaCa-2 cells, and then a series of *in vitro* assays were performed. (A) The silencing effect was confirmed by quantitative real-time PCR, and results indicated that the two shRNA oligos could inhibit HMGB2 expression. (B) Immunoblotting with anti-HMGB2 antibody further validated the silencing effect. (C) CCK-8 proliferation assay demonstrated HMGB2 knockdown could effectively inhibit pancreatic cancer cell viability. (D,E) To check the role of HMGB2 in colony formation, colony-formation assay was performed, and results suggested that silencing HMGB2 expression significantly inhibited the clonogenic activity of PANC-1 and MIA PaCa-2 cells.

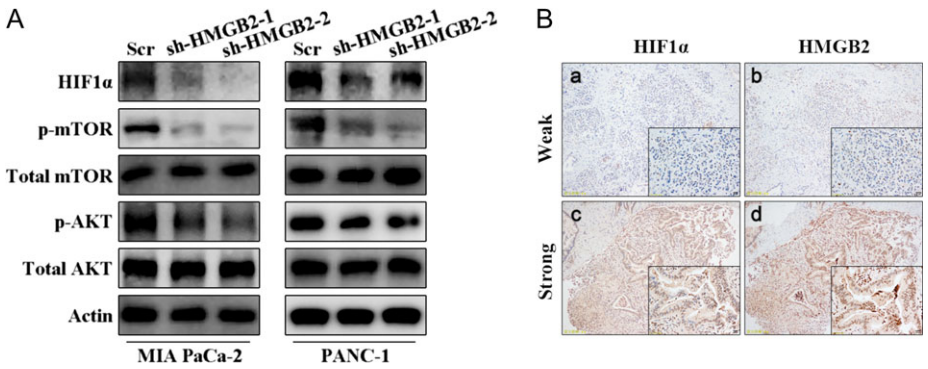

Figure 3. HMGB2 modulates AKT/mTOR/HIF1α signaling pathway AKT/mTOR/HIF1α signaling plays an essential role in cancer cell proliferation and metastasis. To assess whether HMGB2 regulates this pathway, the activation status of the indicated proteins was examined. (A) Silencing HMGB2 decreased phosphorylated AKT and mTOR levels. Moreover, the protein level of HIF1α also decreased in HMGB2-knockdown PANC-1 and MIA PaCa-2 cells. (B) To further validate the correlation between HMGB2 and HIF1α, IHC staining was performed. Results demonstrated that HIF1α protein level was higher in patients with higher HMGB2 expression.

Discussion

In this study, by exploring the TCGA dataset, we investigated the contribution of HMGBs family members to pancreatic cancer prognosis, and validated HMGB2 as a prognostic marker in pancreatic cancer. Our *in vitro* results demonstrated that silencing HMBG2 expression inhibited cell viability and clone formation

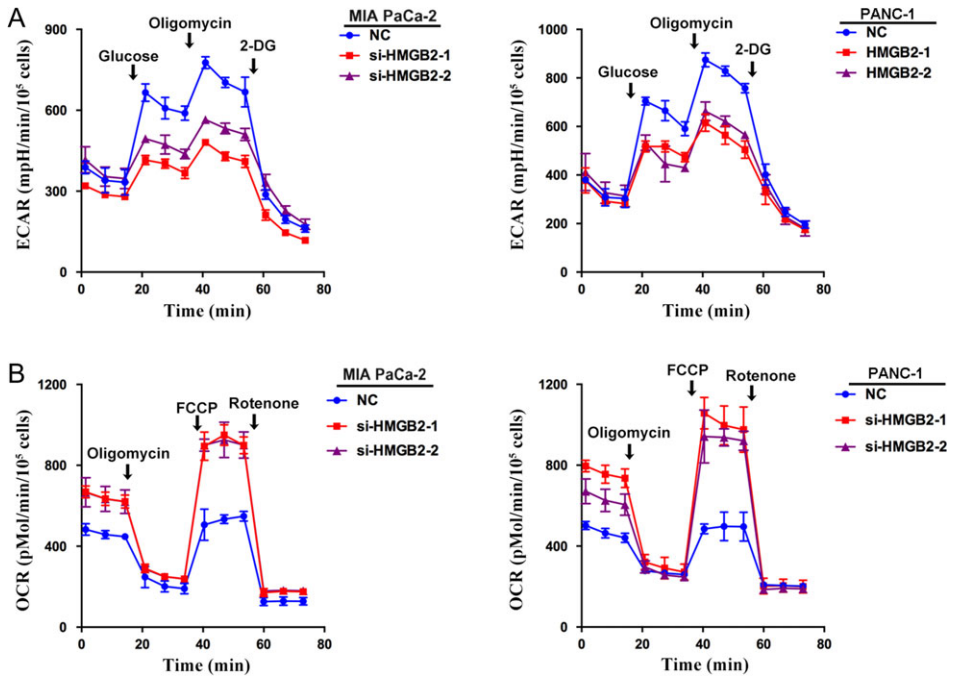

Figure 4. HMGB2 regulates HIF1α-mediated glycolysis in pancreatic cancer cells HIF1α is an important regulator of anabolic glycolysis in pancreatic cancer cells. To check whether HMGB2 promotes the Warburg effect, a series of metabolic assays were performed. (A) Silencing HMGB2 inhibited ECAR value, an indicator that reflects glycolysis in PANC-1 and MIA PaCa-2 cells. (B) In glycolysis, the mitochondrial respiration was inhibited. OCR is an indicator of mitochondrial respiration. In consistent with the ECAR results, OCR levels increased in HMGB2-knockdown PANC-1 and MIA PaCa-2 cells.

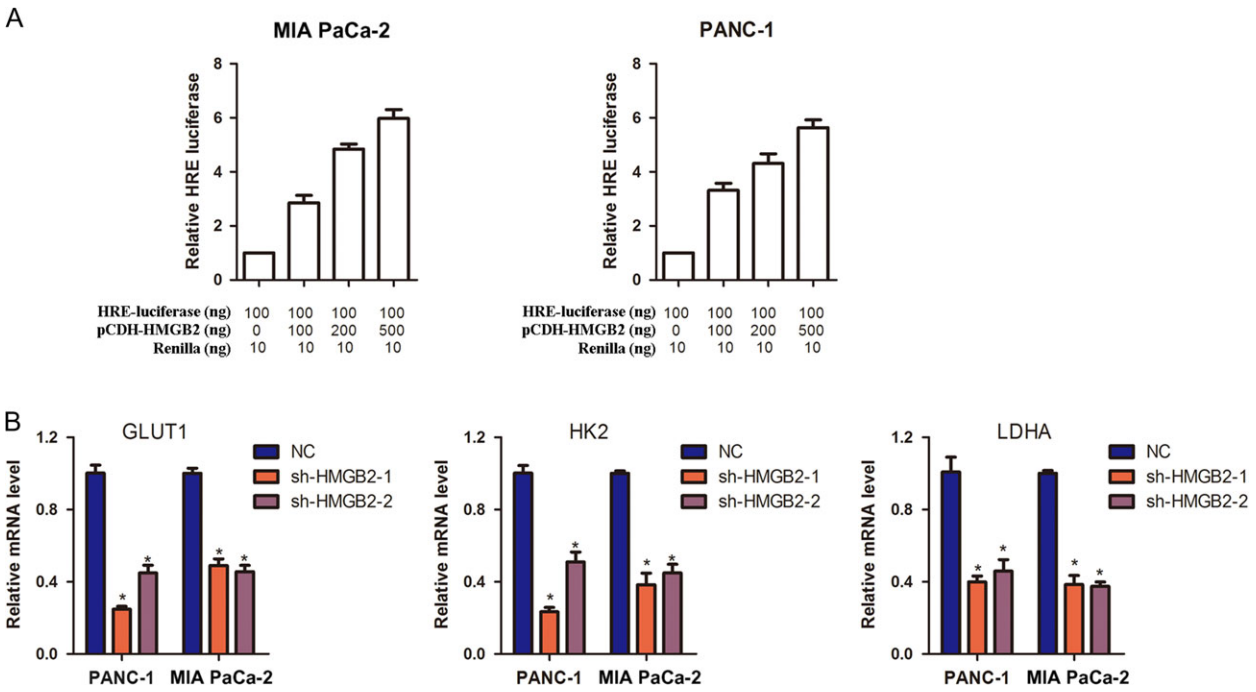

Figure 5. HMGB2 is a positive regulator of *HIF1α* transcription HIF1α regulates glycolysis by regulating a cascade of glycolytic genes, which possess HRE in their promoter region. HRE-luciferase activity reflects the transcription activity of HIF1α. (A) HMGB2 increased the HRE-luciferase activity in PANC-1 and MIA PaCa-2 cells. (B) Silencing HMGB2 decreased the expression levels of *GLUT1*, *HK2*, and *LDHA* in PANC-1 and MIA PaCa-2 cells.

capacity. To explore the underlying mechanism, we analyzed the regulatory function of HMGB2 in anabolic metabolism, a process that sustains cancer cells for uncontrolled proliferation and progression. As demonstrated, silencing HMGB2 inhibited the Warburg effect in pancreatic cancer cells. Moreover, the expressions of relevant glycolytic genes including *GLUT1*, *HK2*, and *LDHA*

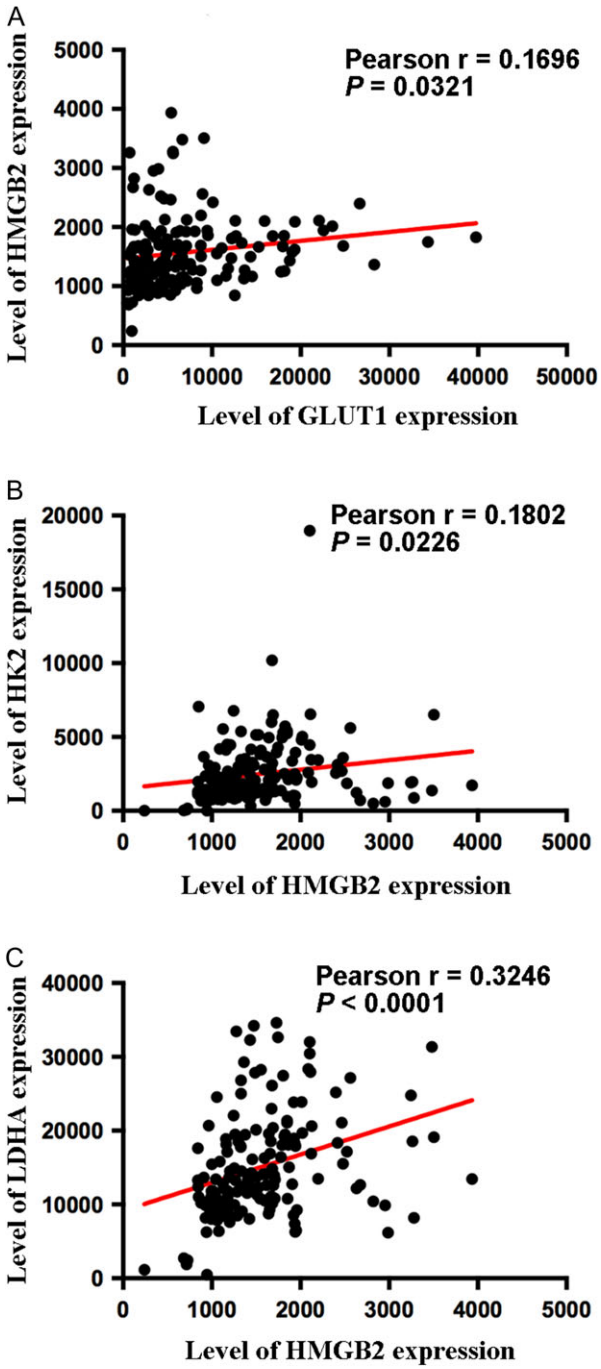

Figure 6. HMGB2 expression is positively correlated with GLUT1, HK2, and LDHA in pancreatic cancer patients To further confirm the contribution and correlation between HMGB2 and glycolytic genes expressions, the expressional levels of these genes were analyzed. (A) HMGB2 was strongly correlated with GLUT1 expression. (B) The expressions of HMGB2 and HK2 exhibited a positive correlation. (C) HMGB2 expression was positively correlated with LDHA in the TCGA dataset.

were decreased in HMGB2-knockdown pancreatic cancer cells. Further validation of the correlation between HMGB2 expression with GLUT1, HK2, and LDHA in the TCGA cohort supported the *in vitro* observations.

HMGBs may form complexes with other proteins, and bind to other receptors in synergy with endogenous and exogenous danger signals to regulate a series of gene expression. For example, HMGB1 possesses the function of promoting cytokine release by interacting with TLR4, thereby promoting cell migration [4]. In this study, our results demonstrated that HMGB2 is a positive regulator of HIF1α-mediated transcription process by directly targeting the AKT1/mTOR/HIF1α, but the exact mechanism remains elusive. Based on the intrinsic function of HMG family members in facilitating transcription factors for binding to chromatin, it is reasonable to speculate that HMGB2 may be an interaction partner of HIF1α [28]. By interaction with HIF1α, HMGB2 facilitates HIF1α in binding to its downstream targets, which may provide the direct mechanism to explain how HMGB2 regulates GLUT1, HK2, and LDHA

expression. Further screening for HMGB2 interaction partners using mass spectrometry is required to further explore the function of HMGB2 in pancreatic cancer.

Another reason for poor outcome of pancreatic cancer is the resistance to traditional therapies, including chemotherapy and radiotherapy. Activation of HIF1 signaling is observed in a broad range of human cancers due to tumor hypoxia brought about by limited blood vessel and reduced oxygen and nutrient supply. HIF1 activation leads to transcription of a series of target genes that promote physiological changes associated with chemotherapy and radiotherapy resistance, including the inhibition of apoptosis and senescence, and even the activation of drug efflux and cellular metabolism [28]. In recent years, the contribution of aberrant cancer metabolism to chemotherapy and radiotherapy has received much attention [29,30]. For example, SIRT4, a mitochondrial localized metabolism regulator, participates in the genotoxic stress regulation, thereby acts as a target for improving drug resistance in cancers [31]. Moreover, glycolytic genes, including *GLUT1*, *HK2*, and *LDHA*, have all been reported to contribute to drug resistance in cancer cells [32–35]. In this study, we demonstrated that HMGB2 is a positive regulator of anabolic mechanism in pancreatic cancer. Based on the contribution of aberrant metabolism to chemotherapy and radiotherapy resistance, it is possible that silencing HMGB2 functions may contribute to the improvement of treatment. Further studies are required to use HMGB2 as an intervention target for improving pancreatic cancer treatment.

In summary, we demonstrated HMGB2 as a poor prognosis factor in pancreatic cancer, and patients with higher levels of HMGB2 exhibited a poor outcome. *In vitro* assays showed that HMGB2 silencing inhibited glycolysis and the expressions of relative genes including *GLUT1*, *HK2*, and *LDHA*. The observation was further validated by examination of the expression correlation in the TCGA dataset. Our results provide novel prediction and treatment targets for pancreatic cancer.

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