Upregulation of macrophage migration inhibitory factor promotes tumor metastasis and correlates with poor prognosis of pancreatic ductal adenocarcinoma

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Abstract. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that serves important roles in cancer. MIF overexpression is frequently observed in numerous human cancer types, including pancreatic carcinoma. However, the prognostic value and function of MIF in pancreatic ductal adenocarcinoma (PDAC) have not been fully elucidated. In the present study, upregulation of MIF expression in PDAC tissue compared with adjacent normal tissue was observed. Furthermore, MIF overexpression was identified to be significantly associated with poor survival rates in patients with PDAC. Multivariate Cox regression analysis confirmed that MIF was an independent risk factor for poor survival. Functional analyses demonstrated that MIF knockdown significantly inhibited the proliferation and invasion of pancreatic cancer cells in vitro compared with control cells. In addition, mechanistic investigations revealed that silencing MIF leads to inhibition of AKT serine/threonine kinase and extracellular-signal-regulated kinase activation, and suppression of cyclin D1 and matrix metalloproteinase-2 expression, which may suppress tumor proliferation and invasion. These results highlight the importance of MIF overexpression in PDAC aggressiveness, and indicate that MIF may be a potential therapeutic target for pancreatic cancer.

Introduction

Pancreatic carcinoma is a common type of cancer associated with high mortality rates and is the fourth leading cause of cancer-associated mortality globally (1). Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer. The 5-year overall survival rate for patients with PDAC is ~5% and median survival time is <6 months (2). Surgical removal of the tumor is the most effective and preferred therapy (3). However, PDAC is characterized by invasive growth and early metastasis, often manifesting in advanced clinical stage at presentation and rapid postoperative recurrence, and only one fifth of patients with PDAC are diagnosed early enough to be candidates for surgical resection (4). Furthermore, due to the frequent recurrence of PDAC, the median survival time even among surgically resected cases is <2 years (1). Therefore, an understanding of the mechanisms of PDAC metastasis and the identification of the factors involved in early metastasis are required to improve treatment and disease outcomes in PDAC.

PDAC is associated with significant intra- and peritumoral inflammation (5). Chronic pancreatitis is a risk factor for PDAC, and new-onset pancreatitis is a common symptom of PDAC. Upregulation of inflammation-associated signaling modulates PDAC progression and therapeutic resistance by inducing proliferation and metastasis, and by suppressing apoptosis (6,7). Macrophage migration inhibitory factor (MIF) is a pleiotropic inflammatory cytokine that is associated with carcinogenesis (8). Through autocrine or paracrine signaling, MIF interacts with cluster of differentiation (CD)74, its primary receptor, and C-X-C chemokine receptor type 4, its co-receptor, to activate the AKT serine/threonine kinase (AKT) and extracellular-signal-regulated kinase (ERK) pathways (9,10). Previous studies from different groups have identified an increased expression level and tumor-promoting functions of MIF in PDAC (11-14). MIF knockdown inhibits...
ERK1/2 and AKT phosphorylation, and upregulates p53 expression, in turn leading to cell cycle arrest and apoptosis in pancreatic cancer cells (11,12). Recently, Yang et al (13) reported a novel signaling pathway whereby MIF upregulates miR-301b, which subsequently targets nuclear receptor subfamily 3 group C member 2. Inhibition of this signaling axis may reduce metastasis and prolong survival in a mouse model of PDAC (13). Furthermore, MIF induces the epithelial to mesenchymal transition and invasion of PDAC cells (13,14). Nevertheless, the role of MIF in pancreatic cancer is not clearly defined.

In the present study, MIF were demonstrated to enhance cyclin D1 and matrix metalloproteinase (MMP)-2 expression by activating AKT and ERK signaling; subsequently promoting metastasis of PDAC cells. We also show that upregulation of MIF is a frequent event in PDAC, and correlates with unfavorable prognosis in pancreatic cancer.

Materials and methods

PDAC tissue samples. The present study was approved by the Human Research Ethics Committees of the First Affiliated Hospital of Sun Yat-sen University (approval no. 201515; Guangzhou, China), according to the Declaration of Helsinki. All of patients enrolled in the present study provided written informed consent.

Human PDAC tissues were collected from 85 patients who underwent resection at the First Affiliated Hospital of Sun Yat-sen University between 2003 and 2007. Patients did not receive any local or systemic chemotherapeutic treatments prior to the surgery. Tumor histopathology was independently classified according to the World Health Organization Classification of Tumors by two pathologists (15). All patients were followed postoperatively to assess survival outcomes. The relevant characteristics of patients are listed in Table I.

Immunohistochemistry (IHC). Formalin-fixed paraffin-embedded PDAC tissues were cut into 5 μm sections, processed for antigen retrieval by pressure cooking in 10 mM citrate buffer (pH 6.0) and blocked with UltraCruz® Blocking reagent (cat. no. sc-516214; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at room temperature for 1 h, followed by incubation at 4°C overnight with rabbit polyclonal antibody against human MIF (cat. no. sc-516214; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Immunostaining was performed with the ChemMate DAKO EnVision Detection kit for Peroxidase/DAB/Rabbit/Mouse (Fisher Scientific, Inc.) RT-qPCR (for cDNA of cell lines).

MIF in PDAC tissues was evaluated under a light microscope at x400 magnification. For each specimen, five images of representative areas were acquired, and a total of 1,000 tumor cells were counted. IHC scoring was performed according to a modified Histo-score (H-score) (16), which includes an assessment of the fraction of positive cells and the intensity of staining. The intensity was assigned a score of 0-3, representing no staining for 0, weak staining for 1, moderate staining for 2, and strong staining for 3. The fraction score was based on the proportion of positively stained cells (0-100%). The intensity and fraction scores were multiplied to obtain an H-score that ranged between 0 and 3, and represented the expression level of MIF protein.

RNA oligoribonucleotides, tumor cell, lines and transfection. Small interfering RNA (siRNA) duplexes that target the human MIF mRNA (si-MIF) were designed using BLOCK-IT™ RNAi Designer software (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and purchased from Guangzhou Ribbio Co., Ltd. (Guangzhou, China). Negative control RNA duplexes (NC) for siRNA were not homologous to any known human sequence. The nucleotide sequences of si-MIF and NC are as follows: si-MIF sense, 5'-GGGUCUACUAACAUAAUAdTdT-3' and antisense, 5'-UAUAUGAUUGUAGACCCdCdTdT-3'; NC-sense, 5'-UUCUCGACAGGUCAGCAGUdTdTdT-3' and antisense, 5'-ACGUGACACGUUCGGAGAGAAdTdTdT-3'.

Human panc-1 and Bxpc-3 pancreatic cancer cell lines were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and maintained in a humidified 5% CO2 incubator at 37°C. Human panc-1 pancreatic cancer cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen; Thermo Fisher Scientific, Inc.) and Bxpc-3 pancreatic cancer cell lines were cultured in RPMI-1640 (RPMI; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; HyClone; GE Healthcare Life Sciences, Logan, UT, USA). RNA oligonucleotides were transfected using Lipofectamine RNAiMAX (Invitrogen; Thermo Fisher Scientific, Inc.). A total of 5x104 cells/well were transfected with 50 nM RNA in a 24-well plate.

RNA isolation, reverse transcription-semi-quantitative polymerase chain reaction (RT-sqPCR) and RT-qPCR. Total RNA was isolated from cells or frozen tumor tissues using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. RNA concentration and quality were evaluated according to spectrometric determination at 260 and 280 nm. A total of 2 μg of total RNA was subjected to DNase I digestion (Fermentas; Thermo Fisher Scientific, Inc.), followed by RT using Moloney murine leukemia virus reverse transcriptase (Promega Corporation, Madison, WI, USA) at 42°C for 1 h followed by termination at 75°C for 5 min. Then, mRNA levels of MIF, cyclin D1, and MMP-2 were analyzed by RT-sqPCR (for cDNA of PDAC tissues and cell lines) or by SYBR-Green (cat. no. A25742; Applied Biosystems; Thermo Fisher Scientific, Inc.) RT-qPCR (for cDNA of cell lines). The specific primers used to amplify the MIF, cyclin D1, or MMP-2 genes and the housekeeping GAPDH gene were as follows: MIF forward, 5'-GAGAGACCCTCCTCAGCA-3' and reverse, 5'-GGCCTCTTAGCGCAAGTGGA-3'; Cyclin D1 forward, 5'-GCTGCCTCCTTGAGAAACGC-3' and reverse, 5'-CACAGGGGCCCAAGGGTC-3'; MMP-2 forward, 5'-AGAGTGCTGATAGACACAGC-3' and reverse, 5'-GGTCAGGTATTGCTAGTCT-3'; GAPDH forward, 5'-GAGTCACGGATTTTGCTGCT-3' and reverse, 5'-GAC AACCTCCGTCTGACAG-3'. GAPDH expression served as an internal control. For RT-sqPCR, the cDNA was amplified as follows: 94°C for 30 sec, 58°C for 30 sec and 72°C for 30 sec for 28 cycles; and a final extension at 72°C for 5 min. The products were resolved on a 1.5% agarose gel and visualized.
Table I. Association between MIF Expression and clinical features.

| Variables          | Case no. | Low | High | P-valuea |
|--------------------|----------|-----|------|----------|
| Sex                |          |     |      | 0.156    |
| Male               | 51       | 22  | 29   |          |
| Female             | 34       | 20  | 14   |          |
| Age, years         |          |     |      | 0.023b   |
| >50                | 69       | 30  | 39   |          |
| ≤50                | 16       | 12  | 4    |          |
| Tumor size, cm     |          |     |      | 0.614    |
| >3                 | 48       | 22  | 26   |          |
| ≤3                 | 37       | 18  | 17   |          |
| Differentiation    |          |     |      | 0.892    |
| Well and moderate  | 52       | 26  | 26   |          |
| Poor               | 33       | 16  | 17   |          |
| TNM                |          |     |      | 0.332    |
| III/IV             | 36       | 20  | 16   |          |
| I/II               | 49       | 22  | 27   |          |
| Lymphatic spread   |          |     |      | 0.036e   |
| No                 | 49       | 29  | 20   |          |
| Yes                | 36       | 13  | 23   |          |
| Hepatic metastasis |          |     |      | 0.750    |
| No                 | 66       | 32  | 34   |          |
| Yes                | 19       | 10  | 9    |          |
| Serum CEA, ng/ml   |          |     |      | 0.022b   |
| ≥10                | 49       | 19  | 30   |          |
| <10                | 36       | 23  | 13   |          |

Analysis was performed on 85 cases. The MIF level was examined by immunohistochemical staining assay. The association between MIF level and clinical features of patients with PDAC was analyzed by chi-square test. aP<0.05, statistically significant difference. MIF, Macrophage migration inhibitory factor; TNM, tumor node metastasis; CEA, carcinoembryonic antigen.

Statistical analysis. Associations between MIF expression and clinicopathological features were examined using the chi-square test. Overall survival was calculated as the duration between the date of tumor resection and the time of mortality. Patients who were lost to follow-up or succumbed to causes unassociated with PDAC were treated as censored events. Kaplan-Meier estimator plots were constructed, and the differences between groups were analyzed using a log rank test. Univariate and multivariate Cox proportional hazards regression analysis was performed to investigate the association between the MIF expression or clinical characteristics of patients and overall survival. Significant prognostic factors identified using univariate analysis were further evaluated by multivariate Cox regression analysis. All statistical tests were performed using a 7800 fast real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following thermocycling conditions: 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec. The gene expression levels were normalized to the expression of GAPDH to calculate the 2−ΔΔCq value (17).

Immunoblotting assay. Proteins from cells or frozen tumor tissues were extracted with RIPA buffer (Invitrogen; Thermo Fisher Scientific, Inc.) and quantified using a bicinchoninic acid assay. The total protein (20 µg/lane) was separated on a 12% polyacrylamide gel, and transferred to a methanol-activated PVDF membrane (EMD Millipore, Billerica, MA, USA). The membrane was blocked in Tris-buffered saline-Tween-20 (TBST) containing 5% bovine serum albumin (Guangzhou Jetway Biotech Co., Ltd., Guangzhou, China) at room temperature for 1 h, then subsequently immunoblotted with primary rabbit polyclonal antibodies against MIF (cat. no. sc-20121; Santa Cruz Biotechnology, Inc.), cyclin D1 (cat. no. 2978; Cell Signaling Technology, Inc., Beverly, MA, USA), MMP-2 (cat. no. 40994; Cell Signaling Technology, Inc.) or GAPDH (cat. no. BM1623; Wuhan Boster Biological Technology, Ltd., Wuhan, China) at 4˚C overnight and then with the secondary antibody (anti-rabbit IgG horseradish peroxidase-conjugated; cat. no. 7074; Cell Signaling Technology, Inc.) at room temperature for 1 h. All the primary antibodies were diluted at a ratio of 1:1,000 and the secondary antibody was diluted at a ratio of 1:5,000. Protein bands were visualized using Clarity Western ECL substrate (Bio-Rad Laboratories, CA, USA). The intensity of each band was densitometrically quantified using ImageJ software (version 1.0; National Institutes of Health, Bethesda, MD, USA).

Cell cycle analysis. Cell cycle analyses were performed using a detergent containing hypotonic solution (Krishan's reagent) containing 10 µg/ml propidium iodide (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) according to the manufacturer's protocol and fluorescence-activated cell sorting (FACS) with a Gallios flow cytometry system (Beckman Coulter, Inc., Brea, CA, USA) assay. Data were analyzed using Kaluza software (version 1.5a; Beckman Coulter, Inc.). Nuclear debris and overlapping nuclei were gated out.

In vitro tumor cell invasion assay. Tumor cell invasion was analyzed in 24-well Boyden chambers with 8-µm pore size polycarbonate membranes (Corning Incorporated, Corning, NY, USA). The membranes were coated with 60 µg of Matrigel (cat. no. 3432-005-01; R&D Systems, Inc., Minneapolis, MN, USA) to form the matrix barrier. Pancreatic cancer cells transfected with NC or si-MIF were resuspended in 100 µl serum-free DMEM 36 h post-transfection, and were added to the upper compartments of the chambers. The lower compartments were filled with 600 µl DMEM or RPMI-1640 containing 10 µg/ml propidium iodide (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) according to the manufacturer's protocol and fluorescence-activated cell sorting (FACS) with a Gallios flow cytometry system (Beckman Coulter, Inc., Brea, CA, USA) assay. Data were analyzed using Kaluza software (version 1.5a; Beckman Coulter, Inc.). Nuclear debris and overlapping nuclei were gated out.

Statistical analysis. Associations between MIF expression and clinicopathological features were examined using the chi-square test. Overall survival was calculated as the duration between the date of tumor resection and the time of mortality. Patients who were lost to follow-up or succumbed to causes unassociated with PDAC were treated as censored events. Kaplan-Meier estimator plots were constructed, and the differences between groups were analyzed using a log rank test. Univariate and multivariate Cox proportional hazards regression analysis was performed to investigate the association between the MIF expression or clinical characteristics of patients and overall survival. Significant prognostic factors identified using univariate analysis were further evaluated by multivariate Cox regression analysis. All statistical tests were performed using a 7800 fast real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following thermocycling conditions: 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec. The gene expression levels were normalized to the expression of GAPDH to calculate the 2−ΔΔCq value (17).
were two-tailed, and P<0.05 was considered to indicate a statistically significant difference. Analyses were performed using SPSS software (version 19.0; IBM Corp., Armonk, NY, USA).

Data are expressed as the mean ± standard error of the mean from three independent experiments. Differences between the groups were analyzed by Student's t-test or one-way analysis of variance with Bonferroni's post hoc test. Analyses were performed with GraphPad Prism, version 5 (GraphPad Software, Inc., San Diego, CA, USA).

**Results**

**Overexpression of MIF is a frequent event in PDAC tissue.** It has been reported that MIF is overexpressed in pancreatic cancer (11,18). To further confirm this, MIF expression was evaluated in 85 paired PDAC and adjacent noncancerous tissues by IHC. MIF expression was observed in 75/85 tumor samples (Fig. 1A). Compared with paired noncancerous tissue, the majority (75/85) of PDAC tumor tissue exhibited significantly higher MIF expression (Fig. 1B). Consistent with the results from IHC analysis, RT-qPCR and immunoblotting assays revealed a similar trend with increased MIF expression in PDAC tissue at the mRNA and protein levels (Fig. 1C). These data indicate that MIF overexpression is prevalent in PDAC.

**Increased MIF expression is associated with poor survival of patients with PDAC.** Whether MIF overexpression is associated with clinical features or clinical outcome of patients with PDAC was investigated. MIF expression was divided into low- and high-expression groups, based on median expression (cut-off value, 1.08) analyzed by IHC in all PDAC cases The associations between the MIF expression and clinical features are summarized in Table I. Increased MIF expression was identified to be associated with advanced age, lymphatic spread and increased serum carcinoembryonic antigen levels (Table I). Furthermore, Kaplan-Meier analysis revealed that higher MIF levels were significantly associated with shorter overall survival time of patients with PDAC (P=0.023; Fig. 2). Cox proportional hazards regression analysis was performed to exclude confounder effects. Univariate Cox analysis was first performed to identify factors that may affect the overall survival of patients with PDAC. Higher MIF expression, poor differentiation, high tumor nodal metastasis (TNM) staging and the presence of metastasis were associated with inferior survival times (Table II). Multivariate Cox analysis adjusted for differentiation grade and TNM stage further confirmed that MIF overexpression was an independent risk factor for poor overall survival in patients with PDAC (Table II). These data suggest that overexpression of MIF may predict the poor survival and may contribute to PDAC metastasis.

**Knockdown of MIF suppresses proliferation and invasion of PDAC cells.** The association of MIF expression with clinical features and poor outcomes for patients with PDAC prompted an investigation into the potential role of MIF in PDAC growth and metastasis. Thus, the potential role of MIF in the regulation of G1/S transition and in vitro invasion of two PDAC cell lines, Panc-1 and Bxpc-3, was examined. Cells were transfected with si-MIF or with si-NC, and were then subjected to a FACS or Boyden chamber Transwell invasion assay. RT-qPCR and immunoblotting assays demonstrated that si-MIF transfection markedly decreased the mRNA and
protein levels of MIF in pancreatic cancer cells (Fig. 3A). Notably, the knockdown of MIF resulted in a marked accumulation of the G1-population in PDAC cells (Fig. 3B and C). Furthermore, si-MIF-transfected Panc-1 and Bxpc-3 cells exhibited a significantly reduced number of cells invading through the Transwell chamber (Fig. 3D and E).

Knockdown of MIF inhibits the activation of AKT and ERK, and suppresses the expression of cyclin D1 and MMP-2. The molecular mechanisms underlying the metastasis-promoting effects of MIF were investigated. The function of MIF is associated with two major tumor-promoting signaling pathways, namely the AKT and ERK signaling pathways (9,10). Consistently, silencing MIF in PDAC cells significantly decreased the phosphorylation levels of AKT and ERK, but had no effect on the expression of total AKT and ERK protein (Fig. 4A and B), indicating that MIF knockdown inhibited the activation of AKT and ERK signaling in PDAC cells. Cyclin D1 and MMP-2 are important factors upregulated by AKT and ERK signaling that execute AKT- and ERK-mediated cell invasion (19-22). The mRNA and protein levels of cyclin D1 and MMP-2 were significantly reduced in si-MIF-transfected cells compared with the NC group (Fig. 4C and D). These data suggest the MIF enhances PDAC metastasis by activating the AKT and ERK pathways, which in turn upregulates the expression of cyclin D1 and MMP-2.

Discussion

PDAC manifests as a highly aggressive cancer with poor prognosis. The identification of molecules that are involved in PDAC tumor progression and aggressiveness may elucidate novel targets for PDAC treatment. Previous studies have suggested that MIF, a pro-inflammatory cytokine, facilitates cancer progression, associating inflammation with pancreatic cancer progression (12-14,23). Emerging research has explored the biological effects of MIF in PDAC (11-14,23). Similar to
other reports that have described a regulatory function of MIF in cell growth and survival (24-29), pro-proliferation and anti-apoptosis roles for MIF in PDAC have been identified (11). Furthermore, it has been reported that MIF induces epithelial to mesenchymal transition and enhances tumor aggressiveness in PDAC (13,14). Additionally, exosome-derived MIF may prime the liver for PDAC metastasis, and may be a potential biomarker for liver metastasis (23). Consistently, a pro-metastasis effect was observed regarding MIF in PDAC in present study. Taken together, these data highlight the importance
of MIF overexpression in promoting PDAC progression, and suggest that inhibition of MIF may offer a novel therapeutic option for treatment of PDAC.

Although MIF overexpression has been observed in patients with PDAC, to the best of our knowledge, only one report has explored the associations between MIF expression,
increased tumor expression of MIF and decreased survival rates in patients with PDAC following tumor resection (14). In the present study, the overexpression of MIF was demonstrated to be a frequent event in PDAC. Notably, the peritumoral tissues of PDAC expressed undetectable or low levels of MIF protein in acinar and ductal cells as well as stromal cells. As known, PDAC is associated with intra- and peritumoral inflammation, which may significantly induce MIF expression in peritumoral tissues (30). The variation in MIF expression between the peritumoral tissues of PDAC samples may be at least partly due to the differing degree of peritumoral inflammation. The results of the present study demonstrated that high levels of MIF were associated with metastasis and inferior survival of patients with PDAC, and that high MIF expression may serve as an independent risk factor for poor disease outcome. Using an siRNA-based strategy, the pro-metastasis effect of endogenous MIF expression was examined in human PDAC cell lines, further supporting the role for MIF in PDAC aggressiveness.

PDAC has a high tendency to metastasize. Typically, PDAC cells first spread to nearby lymph nodes, and later metastasize to the liver and other organs. Thus, lymphatic spread is a critical early event of PDAC progression. Consistently, the present study demonstrated that lymphatic spread and hepatic metastasis are significantly associated with poor survival of patients with PDAC. The association between high MIF expression and lymphatic spread indicated that MIF may be a possible mediator of extensive lymph node metastasis. Furthermore, the finding that silencing of MIF inhibits the invasion of PDAC cells in vitro supports the possibility that MIF facilitates lymph node metastasis of PDAC cells. Nevertheless, this hypothesis requires further evaluation using additional in vivo studies.

The present study results revealed that that silencing of MIF significantly inhibited the expression of MMP-2 and CCND1, suggesting that MMP-2 and CCND1 may be target genes that mediate the pro-metastasis effects of MIF. MMP-2 is a member of the matrix metalloproteinase family, is frequently overexpressed in tumors, and is well known to facilitate invasion and metastasis of tumor cells by degrading extracellular matrix (31). CCND1, a regulator of cell cycle (32,33), promotes tumor invasion and metastasis, according to evidence from clinical studies and in vivo experiments (34,35). On one hand, nuclear CCND1 and its binding partner Cdk4 act as a transcriptional regulator of genes controlling cell adherence and migration (36,37). On the other hand, CCND1 in the cytoplasm phosphorylates cytoplasmic and membrane-associated proteins to promote cell spreading and invasion (38). Notably, we previously reported that MIF promotes hepatocellular carcinoma cell growth by positively regulating CCND1 expression (26). The data from cell cycle analysis also revealed that silencing of MIF blocked G1/S transition of PDAC cells (data not shown), indicating that MIF promoted PDAC cell cycle by inducing CCND1 expression. Taken together with the results of the present study, these data indicate that CCND1 may serve a central role in MIF-mediated tumor progression.

In conclusion, the findings of the current study identified a MIF/AKT/ERK/CCND1/MMP-2 cascade that promotes PDAC metastasis. The results suggest that MIF is a candidate prognostic indicator in patients following resection of PDAC. Additional pre-clinical studies are necessary to evaluate the effect of MIF-targeting in pancreatic cancer as a novel therapeutic approach.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors' contributions
WL and JTX designed the study. DW, RW, AH and ZF performed the experiments. DW, KW and MH analyzed the experimental data. AH and MH collected the tissue specimens. WL, JTX, DW and RW wrote the manuscript. All authors discussed the results and contributed to the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The present study was approved by the Human Research Ethics Committees of the First Affiliated Hospital of Sun Yat-sen University (approval no. 201515; Guangzhou, China), according to the Declaration of Helsinki. All of patients enrolled in the present study provided written informed consent.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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