Cancer Research

Hypoxia-Inducible Factor-1 Promotes Pancreatic Ductal Adenocarcinoma Invasion and Metastasis by Activating Transcription of the Actin-Bundling Protein Fascin

Xiao Zhao[1], Song Gao[1], He Ren[1], Wei Sun[1], Huan Zhang[1], Jianwei Sun[2], Shengyu Yang[2], and Jihui Hao[1]

Abstract

Because of the early onset of local invasion and distant metastasis, pancreatic ductal adenocarcinoma (PDAC) is the most lethal human malignant tumor, with a 5-year survival rate of less than 5%. In this study, we investigated the role of fascin, a prometastasis actin-bundling protein, in PDAC progression, invasion, and the molecular mechanisms underlying fascin overexpression in PDAC. Our data showed that the expression levels of fascin were higher in cancer tissues than in normal tissues, and fascin overexpression correlated with the PDAC differentiation and prognosis. Fascin overexpression promoted PDAC cell migration and invasion by elevating matrix metalloproteinase-2 (MMP-2) expression. Fascin regulated MMP-2 expression through protein kinase C and extracellular signal—regulated kinase. Importantly, our data showed that hypoxia induced fascin overexpression in PDAC cells by promoting the binding of hypoxia-inducible factor-1 (HIF-1) to a hypoxia response element on the fascin promoter and transactivating fascin mRNA transcription. Intriguingly, HIF-1α expression levels in PDAC patient specimens significantly correlated with fascin expression. Moreover, immunohistochemistry staining of consecutive sections demonstrated colocalization between HIF-1α and fascin in PDAC specimens, suggesting that hypoxia and HIF-1α were responsible for fascin overexpression in PDAC. When ectopically expressed, fascin was able to rescue PDAC cell invasion after HIF-1α knockdown. Our results demonstrated that fascin is a direct target gene of HIF-1. Our data suggested that the hypoxic tumor microenvironment in PDAC might promote invasion and metastasis by inducing fascin overexpression, and fascin might be targeted to block PDAC progression. *Cancer Res; 74(9); 2455–64. ©2014 AACR.*

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies with a 5-year survival rate of less than 5% (1). The poor prognosis in PDAC is mainly due to early onset of local invasion and distant metastasis. Therefore, it is important to understand the cellular and molecular mechanisms that regulate tumor invasion and metastasis to develop better treatment regimens.

Authors' Affiliations: [1]Department of Pancreatic Carcinoma, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin, China; and [2]Department of Tumor Biology and Comprehensive Melanoma Research Center, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

X. Zhao and S. Gao contributed equally to this work.

Corresponding Author: Jihui Hao, Department of Pancreatic Carcinoma, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center of Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin 300060, China. Phone: 86-022-23340123; Fax: 86-022-23340123; E-mail: jihuihao@yahoo.com

doi: 10.1158/0008-5472.CAN-13-3009

Hypoxia is most commonly presented in the microenvironment of solid tumors (2). Hypoxia-inducible factor-1 (HIF-1; consists of highly regulated HIF-1α and a constitutively expressed HIF-1β) is the most important transcription factor as a result of intratumoral hypoxia, which can mediate many adaptive physiologic responses (3). In pancreatic cancer, HIF-1α expression levels are associated with tumor progression, fibrotic focus, angiogenesis cell migration, and hepatic metastasis (4–6).

Fascin is an actin-bundling protein that crosslinks actin filaments into tight, parallel bundles in filopodia and invadopodia (7–9). At the cellular level, fascin is highly expressed in filopodia-rich cell types such as neurons, mature dendritic cells (10), but not expressed in the normal simple columnar epithelia of biliary duct, breast, colon, ovary, pancreas, and stomach. However, the absence or low expression of fascin in normal epithelia is dramatically altered in many human carcinomas (11). There is evidence suggesting that fascin is overexpressed in PDAC (12–14), and fascin overexpression resulted in enhanced motility, scattering, and invasiveness of pancreatic cancer cells (15). However, molecular mechanisms underlying fascin overexpression in PDAC is not clear, and the role of fascin in PDAC progression is not fully understood.

In this study, we aimed to investigate (i) the mechanism of fascin regulation by hypoxia, (ii) the role of fascin in the

invasion of PDAC, and (iii) the correlation between HIF-1 and fascin in specimens of pancreatic cancer.

Materials and Methods

Cell culture and hypoxic treatment

293T cells and Aspc-1 human PDAC cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. MiaPaCa-2 cells were from American type culture collection. All of the cells were obtained before 2011, and recently authenticated in January 2013 by the short tandem repeat analysis method using Promega PowerPlex1.2 analysis system (Genewiz Inc.). Cells were grown at 37°C in a humidified atmosphere of 95% air and 5% CO_2, using Dulbecco's Modified Eagle Medium (DMEM) with 10% FBS. For hypoxic treatment, cells were placed in a modulator incubator in an atmosphere consisting of 94% N_2, 5.0% CO_2, and 1%O_2. U0126 (CST) and bisindolymalei mide I (Enzo Life Sciences) were dissolved in dimethyl sulfoxide (10 and 2 mmol/L, respectively).

Immunohistochemistry

Immunohistochemistry for HIF-1α and fascin of PDAC patient tissues was performed according to the manufacturer's instructions, using a DAB Substrate Kit (Maxin). The results were scored by two examiners who were blinded to clinicopathologic data. Intensity of staining was scored as (0 = negative; 1 = low; 2 = medium; 3 = high). Extent of staining was scored as 0 = 0% stained; 1 = 1% to 25% stained; 2 = 26% to 50% stained; 3 = 51% to 100% stained. Five random fields were observed under a light microscope. The final score was determined by multiplying the scores of intensity with the extent of staining, ranging 0 to 9. Final scores of less than 1 were considered as negative staining (−), 1 to 2 as low staining (+), 3 to 4 as medium staining (++), and 6 to 9 as high staining (+++).

siRNA duplexes, plasmid constructs, transient transfection, and luciferase assay

siRNAs against fascin were designed and synthesized from Ribobio (Supplementary Table S1). siRNA against protein kinase C (PKC) was purchased from Santa Cruz Biotechnology (sc-29449). siRNAs against HIF-1α and pcDNA-HIF-1α plasmids were prepared as previously described (4, 16). The full-length fascin cDNA (GenBank no.6624) was ligated into expression vector pEGFP-C1 (Invitrogen) and verified by sequencing.

Genomic DNA fragments of the human fascin gene, spanning from −9 to −1942 relative to the transcription initiation site, were generated by PCR and inserted into pGL3-Basic vectors (nominated pGL3-Fascin-promoter). All constructs were sequenced to confirm their identity. Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) as described (16).

For transfection, cells were plated at a density of 5×10^5 cells/well in 6-well plates containing serum-containing medium. When the cells were 80% confluent, 50 nmol/L siRNAs or 4 μg plasmids were transfected into cells using Lipofectamine-2000 (Invitrogen) for 48 hours.

Real-time quantitative reverse transcription PCR

Total RNA was isolated from cells using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. A reverse-transcription PCR (RT-PCR) system (TaKaRa) was used. Then, 1 mg sample of the cDNA was quantified by real-time PCR using primer pairs with SYBR Green PCR Master Mix (TaKaRa). Each sample was done in triplicate. β-actin was used as loading control. PCR primers used are indicated in Supplementary Table S1.

Western blot analysis

Whole-cell extracts were prepared by lysing cells with SDS lysis buffer supplemented with proteinase inhibitors cocktail (Sigma). Protein lysates (20 mg) were separated by SDS-PAGE, and target proteins were detected by Western blot analysis with antibodies (Supplementary Table S1)

Chromatin immunoprecipitation assay

A chromatin immunoprecipitation assay was performed using a commercial kit (Upstate Biotechnology) according to the manufacturer's instruction. Primers flanking the hypoxia response element (HRE) of the VEGF promoter were used as a positive control. The PCR primers are indicated in Supplementary Table S1.

Wound-healing and invasion assays

A wound-healing assay was performed according to published protocol (17). Invasion assays were performed with 8.0 μm pore inserts in a 24-well Transwell. For this assay, 30,000 cells were isolated and added to the upper chamber of a Transwell with DMEM. The invasion assay was performed using 1/6 diluted Matrigel (BD Biosciences)-coated filters. DMEM with 10% FBS was added to the lower chamber and the cells were allowed to incubate for 24 hours. Invading cells were quantified after hematoxylin and eosin staining. Each experiment was performed in triplicate and mean values are presented.

Statistical analysis

A Student *t* test or ANOVA for unpaired data was used to compare mean values. A Spearman rank correlation coefficient test was carried out for testing the association between ordinal variables. The log-rank test was used to obtain a *P* value for the significance of Kaplan–Meier curves' divergence. All probability values were two sided. Analyses were performed using the SPSS17.0 statistical analysis software. Each experiment was done in triplicate and values are presented as mean + SD.

Results

Fascin protein expression in PDAC tissues

To understand the role of fascin in PDAC progression, we used immunohistochemistry to determine fascin expression levels in a cohort of 79 patients with PDAC treated at the Tianjin Cancer Hospital (Tianjin, China). None of the patients had received any prior antitumor treatment. Fascin signal was detected in most (81%) of the PDAC tissues (Table 1). Intriguingly, normal pancreas ductal epithelium cells adjacent to PDAC tissues were negative for fascin expression (Fig. 1A). In pancreas tumors other than PDAC, such as acinic cell

Table 1. Correlation of fascin expression to clinicopathological features in patients with PDAC

	Fascin			
	− and +	++ and +++	rs	*P*
Sex	—	—	0.081	0.476
Male	25	18	—	—
Female	18	18	—	—
Age, y	—	—	0.109	0.339
<65	35	26	—	—
≥65	8	10	—	—
LN	—	—	0.065	0.570
−	20	14	—	—
+	23	21	—	—
pTNM stage	—	—	0.185	0.104
I	4	0	—	—
II	24	17	—	—
III	8	11	—	—
IV	7	7	—	—
Differentiation	—	—	0.285	0.011
Well	23	10	—	—
Moderate	10	9	—	—
Poor	10	17	—	—
Tumor size (cm)	—	—	0.201	0.085
<5	30	19	—	—
≥5	10	15	—	—
Blood vessel infiltration	—	—	−0.045	0.726
−	21	23	—	—
+	10	9	—	—

NOTE: *P* values were calculated by the Spearman rank correlation test. The numbers of samples vary because clinical data were incomplete in some cases. None of the patients had received any prior antitumor treatment.
Abbreviations: LN, regional lymph node metastasis; pTNM, pathologic TNM stage.

carcinoma and neuroendocrine tumor, fascin expression was negative (Fig. 1A).

Fascin expression in cancer cells was much stronger than dysplasia cells and lowly differentiated cancer cells than in moderately differentiated cancer cells (Fig. 1B), suggesting that fascin expression levels increase with the progression of PDAC. Indeed, we also found that fascin was correlated with the pathologic tumor–node–metastasis (TNM) stage (rs = 0.222, $P = 0.051$) and differentiation (rs = 0.285, $P = 0.011$) of PDAC samples (Table 1). Importantly, patients with PDAC with high or medium (+++ or ++) fascin protein expression had significantly worse overall survival than those with negative or low (− or +) fascin expression ($P = 0.004$, HR = 2.218; Fig. 1C; median survival time: 17 vs. 10 months), suggesting that fascin plays an important role in PDAC progression.

Fascin promotes motility and invasiveness of human PDAC cells through upregulation of MMP-2 expression

To understand the role of fascin in PDAC cells, we used fascin siRNA or overexpression plasmids to examine whether a change in fascin expression affects cancer cell motility. First, we proved the effects of fascin siRNA or overexpression plasmids in MiaPaCa-2 cells. Fascin siRNA (si-fascin#1or2) diminished fascin expression, and fascin overexpression plasmids (pEGFP-fascin) increased EGFP–fascin fusion protein expression (Fig. 2A).

In wound-healing and invasion assays, fascin siRNA decreased the migration and invasion of fascin-silenced cells (Fig. 2B). We also determined the effects of fascin overexpression plasmids on cell invasion and wound healing in addition to siRNA (Fig. 2B). As expected, fascin overexpression significantly enhanced migration and invasion.

We then examined the effect of fascin on expression of some proteins critical for cell invasion and epithelial–mesenchymal transition (EMT), including matrix metalloproteinase (MMP)-2, MMP-9, E-cadherin, fibronectin, and vimentin using Western blotting. As shown in Fig. 2C, fascin knockdown decreased MMP-2 levels (and fascin overexpression increased MMP-2 levels) in MiaPaCa-2 cells without noticeable effects on the expression of other proteins. These results suggested that MMP-2 is involved in fascin-mediated PDAC cell invasion.

To determine how fascin regulates MMP-2 expression, we surveyed the upstream signaling pathways of MMP-2. Several signaling pathways, including PI3K/AKT, extracellular signal—regulated kinase (ERK), and p38, have been previously implicated in the regulation of MMP-2 expression (18–23). We

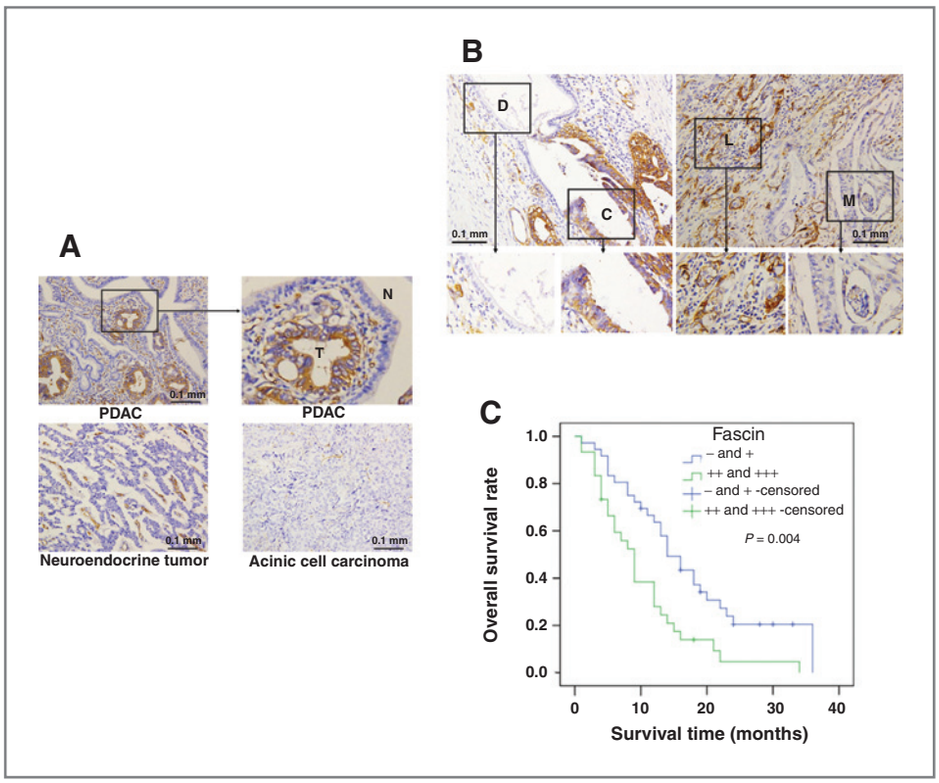

Figure 1. Fascin protein expression in PDAC tissues. A, expression of fascin in PDAC, acinic cell carcinoma, and neuroendocrine tumor tissues, detected using immunohistochemical staining (brown) with hematoxylin and eosin staining by fluorescence microscopy. Magnification, ×200; N, normal; T, tumor. B, expression of fascin in dysplasia (D) cells and cancer (C) cells, lowly (L) and moderately (M) differentiated PDAC cells. Magnification, ×200. C, association between fascin expression levels and the overall survival of patients with PDAC. Patients with PDAC ($n = 79$) were stratified into two groups according to fascin IHC staining intensity. Patients with high fascin expression (intensity grade ++ and +++) had much worse overall survival when compared with patients with low fascin expression (intensity grade − and +). $P = 0.004$ was determined with a log-rank test.

determined the phosphorylation levels of these signaling pathways by Western blot analysis after transfected with fascin siRNAs or overexpression plasmids. Only the phosphorylation level of ERK had changed with the rise and fall of fascin expression (Fig. 2D).

We then examined whether fascin regulates MMP-2 expression through ERK signaling. We transfected fascin-containing plasmids (pEGFP-fascin) into MiaPaCa-2 cells, and then blocked ERK signaling in these cells using its specific inhibitor (U0126, 10 μmol/L). Fascin-overexpressing cells showed an increased level of MMP-2 expression, which was diminished by blocking of ERK signaling (Fig. 2E). An inhibitory effect of blocking of ERK signaling on cell invasion by fascin overexpression also was detected (Fig. 2E).

In an effort to determine the mechanism by which fascin activates ERK, we found that PKC was involved. It was previously reported that activated PKC could bind to and phosphorylate fascin at Ser 39 to inactivate fascin (24); however, the effects of fascin on PKC activity are not known. To examine the hypothesis that fascin might regulate ERK activity through PKC, we investigated the effects of fascin overexpression on PKC activity in MiaPaCa-2 cells. As shown in Fig. 2F, the activity of PKC significantly increased after overexpressing fascin. PKC could regulate ERK activation in various cells (25–27). To explore the role of fascin-induced increase of PKC activity, we used 0.02 μmol/L bisindolymalei mide I, a specific inhibitor of PKC or siRNA against PKC. After transfecting fascin overexpression plasmids, level of phosphorylation of ERK increased, which was reduced by inhibition of PKC (Fig. 2F). These results suggest that fascin enhanced phosphorylation of ERK through upregulating activity of PKC in MiaPaCa-2 cells and thereby increased MMP-2 expression.

HIF-1α regulated the expression of fascin in PDAC cells, and affected the invasion of PDAC cells through fascin

Because hypoxia is commonly presented in the microenvironment of solid tumors, it is important to determine whether hypoxia promotes the expression of fascin in PDAC cells. First, fascin expression was determined by quantitative real-time RT-PCR (qRT-PCR) on MiaPaCa-2 and Aspc-1 cells cultured under normoxia (21% O_2) or hypoxia (1% O_2) for 12 hours. As shown in Fig. 3A, compared with normoxia, fascin expression was increased by 2.1- and 3.8-folds under hypoxia in Aspc-1 and MiaPaCa-2 cells. As a positive control, VEGF expression was increased, too. Western blot analysis showed significant induction of fascin, with the upregulation of HIF-1α protein under hypoxia for 12 hours (Fig. 3A).

To identify the function of HIF-1α in hypoxia-induced expression of fascin in PDAC cells, we used specific siRNA to knockdown HIF-1α. We found that knockdown of HIF-1α expression dramatically decreased the expression of fascin under hypoxia (Fig. 3B), suggesting that HIF-1α is required for hypoxia-induced overexpression of fascin in PDAC cells.

We then examined whether HIF-1α regulates cell invasion through fascin expression through HIF-1α knockdown and fascin rescue. HIF-1α knockdown decreased the expression of fascin protein, which was rescued by transfection with fascin overexpression plasmids (Fig. 3C). We also performed cell invasion assays on these cells. As shown in Fig. 3C, HIF-1α knockdown decreased the invasion of MiaPaCa-2 cells by

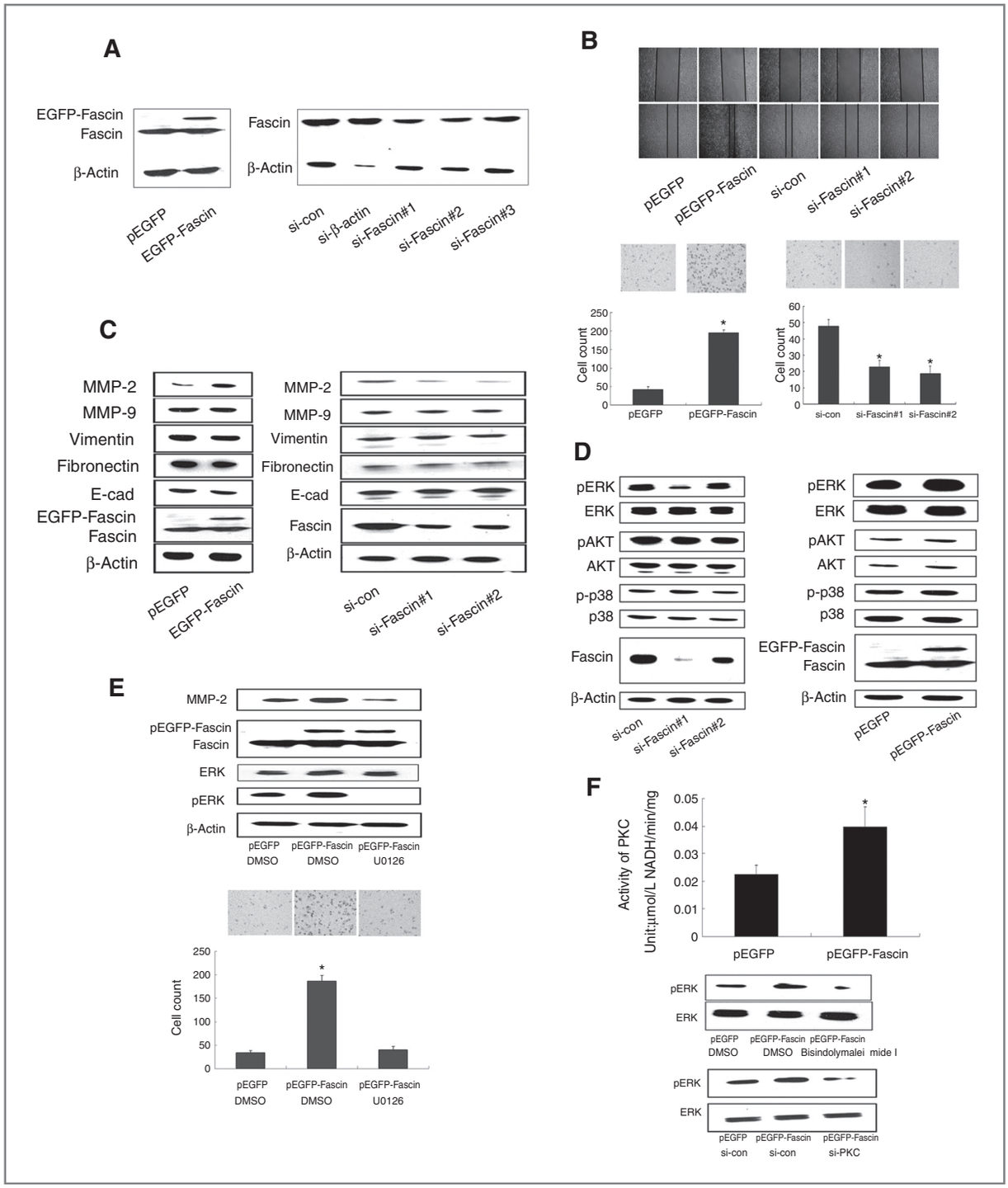

Figure 2. Fascin promotes motility and invasiveness of human PDAC cells through upregulation of MMP-2 expression. A, fascin protein expression in MiaPaCa-2 cells transfected with normal control siRNA (si-con), fascin siRNA (si-fascin#1-3), pEGFP-C1 (pEGFP), or pEGFP-C1-fascin (pEGFP-fascin) overexpression plasmids determined by Western blotting. β-actin was used as a loading control. B, wound-healing assay and invasion assay of MiaPaCa-2 cells were performed with transfected fascin siRNAs or overexpression plasmids. Results of the invasion assay are shown as a histogram (*, $P < 0.05$). Cell migration was measured by microscopy. Magnification, ×100. C, proteins related with EMT or invasion expression, such as MMP-2, MMP-9, E-cadherin (E-cad), fibronectin, vimentin, in MiaPaCa-2 cells transfected with fascin siRNAs or overexpression plasmids determined by Western blotting. D, Western blot analysis showing related changes of phosphorylation of ERK in response to transfected fascin siRNAs or overexpression plasmids in MiaPaCa-2 cells. E, Western blot and invasion analysis: MiaPaCa-2 cells were transfected with pEGFP-C1 (pEGFP) or pEGFP-C1-fascin (pEGFP-fascin) overexpression plasmids. Cells were incubated in the absence or presence of 10 μmol/L U0126 12 hours before analysis. F, activities of PKC were detected after fascin overexpression plasmids transfection. Western blot analysis of phosphorylation of ERK in MiaPaCa-2 cells that were transfected with fascin overexpression plasmids in the absence or presence of 0.02 μmol/L bisindolymaleimide I 12 hours before analysis or siRNA against PKC.

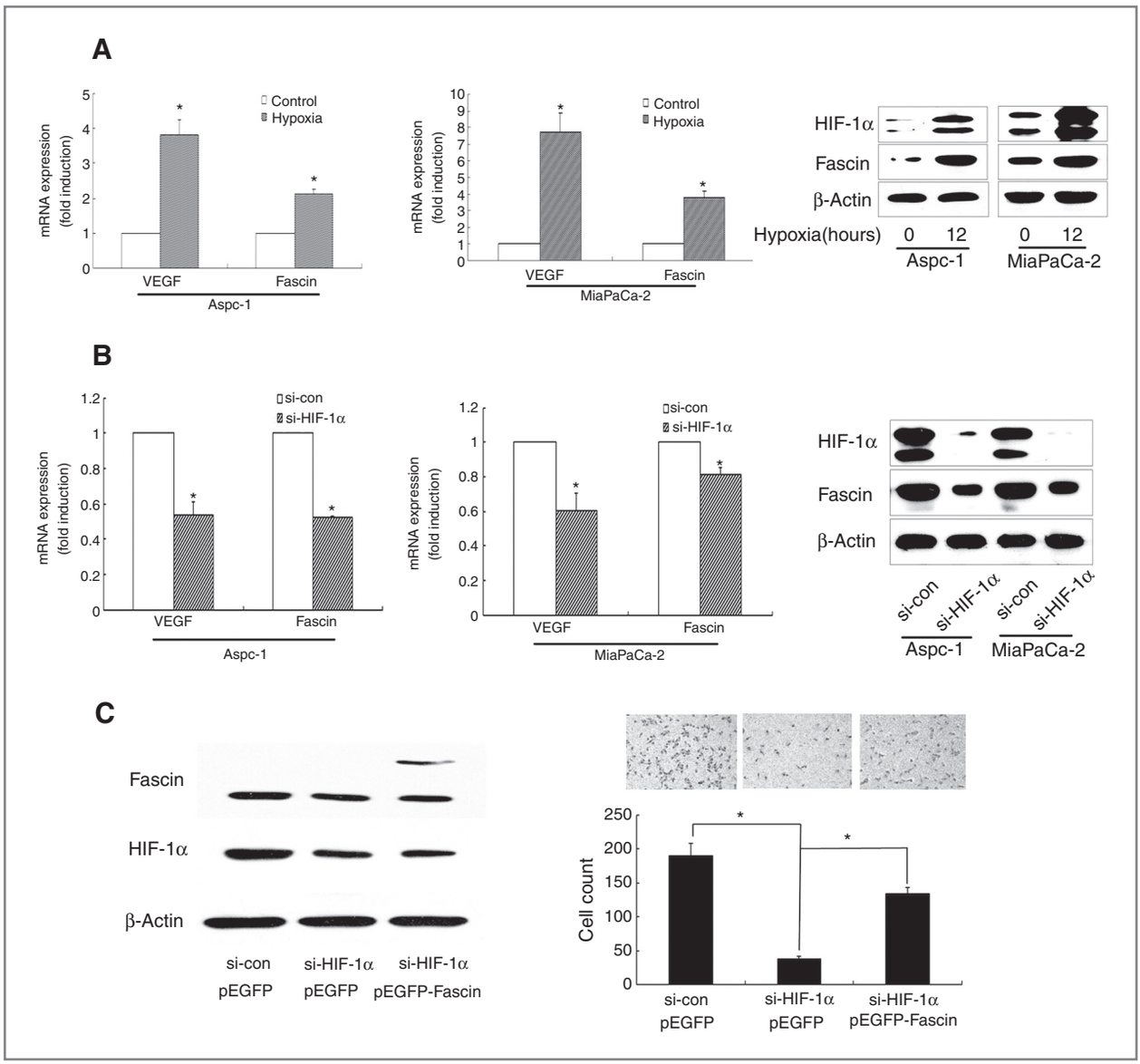

Figure 3. HIF-1α regulated the expression of fascin in PDAC cells and affected the invasion of PDAC cells through fascin. A, Western blot and qRT-PCR analysis in MiaPaCa-2 and Aspc-1 cells cultured under normoxia (21% O_2) or hypoxia (1% O_2) for 12 hours. *, $P < 0.05$ versus control. B, Western blot and qRT-PCR analysis in MiaPaCa-2 and Aspc-1 cells after knockdown of si-HIF-1α (50 nmol/L) under hypoxia condition. *, $P < 0.05$ versus control. β-actin was used as a normalization control. C, expression levels of HIF-1α and fascin were detected by Western blot analysis in MiaPaCa-2 cells, which were transfected with HIF-1α siRNA or scrambled RNA as a negative control, and then followed by transfection with fascin overexpression plasmids. These cells were analyzed by invasion assay, presented as histograms. *, $P < 0.05$.

80.5%, suggesting the critical role of HIF-1α in PDAC invasion. Ectopic expression of fascin in HIF-1α knockdown cells significantly rescued the invasion of HIF-1α knockdown cells, suggesting that HIF-1α regulates PDAC invasion through increasing fascin expression.

HIF-1α upregulated fascin promoter activity

To understand how HIF-1α regulates fascin expression, we surveyed the fascin promoter regions, and identified four potential HIF-1–binding sites (HRE1-4) upstream to the transcription start site (Fig. 4A). To determine whether HIF-1α directly binds to HREs on the fascin promoter, we perform chromatin immunoprecipitation assay in MiaPaCa-2 cells under hypoxia (1% O_2) or normoxia (21% O_2) conditions. The HREs immunoprecipitated by anti-HIF-1α antibodies were detected by PCR. The PCR products of VEGF promoter were used as positive control. As shown in Fig. 4B, the fragments containing HRE3 were significantly increased under hypoxia, suggesting that HIF-1α regulated fascin overexpression by binding to this region.

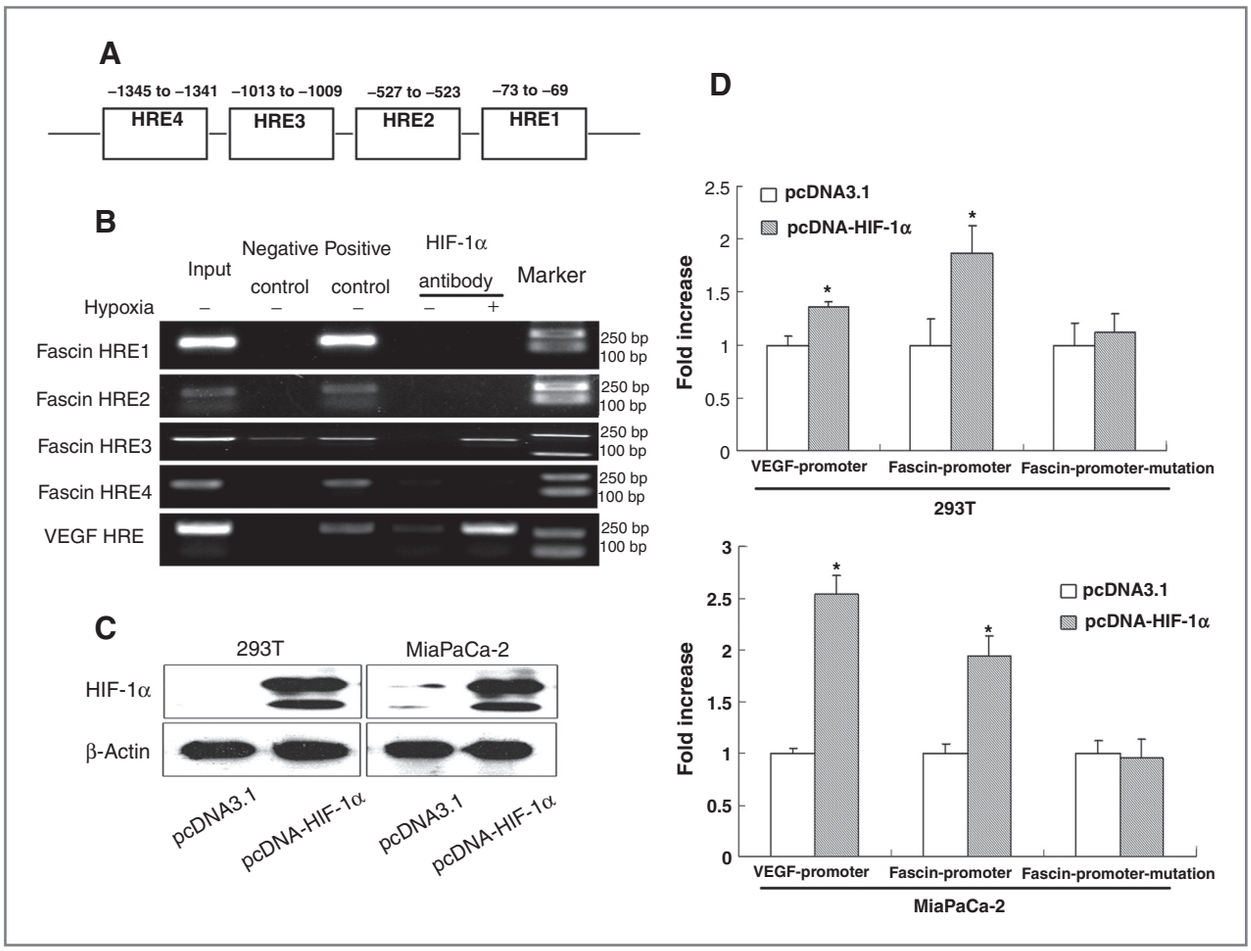

Figure 4. HIF-1α upregulated fascin promoter activity. A, the DNA sequence of the fascin promoter. Four HRE sites were located at a different site. B, chromatin immunoprecipitation analysis in MiaPaCa-2 cells. The PCR products of VEGF promoter were used as positive control. C, Western blot analysis. MiaPaCa-2 and 293T cells were transfected with HIF-1α overexpression plasmids. D, luciferase analysis in MiaPaCa-2 and 293T cells. pGL3-Fascin-promoter: fascin 5′-flanking sequences (–1942/-9) were fused to pGL3 luciferase coding sequence. pGL3-Fascin-promoter-mutation: equal to pGL3-Fascin-promoter, except that HRE3 site was mutated from GCGTG to GCATG. pGL3-VEGF-promoter: VEGF 5′-flanking region (–1948/-7) was fused to pGL3 luciferase coding sequence. pGL3-Basic, empty pGL3 control vector. 293T and MiaPaCa-2 cells overexpressing HIF-1α expression plasmids (pcDNA-HIF-1α) or control vector (pcDNA3.1) were transfected with pGL3-Fascin-promoter, pGL3-Fascin-promoter-mutation, pGL3-VEGF-promoter, or pGL3-Basic. After transfection for 48 hours, cells were subjected to dual luciferase analysis. Results were expressed as a fold induction relative to the cells transfected with the control vector (pcDNA3.1) after normalization to *Renilla* activity. Columns, mean of three independent experiments; bars, SD. *, $P < 0.05$.

To determine whether the binding of HIF-1α is sufficient to transactivate fascin promoter, we constructed a fascin promoter luciferase reporter containing the 5′-flanking sequences (–1942/-9, pGL3-Fascin-promoter) in the fascin promoter and HIF-1α overexpression plasmids containing HIF-1α cDNA fragments (pcDNA-HIF-1α). The 293T cells, which express minimal levels of HIF-1α under normoxic conditions, were used to minimize the interference of endogenous HIF-1α. After cells were transfected with HIF-1α cDNA, HIF-1α protein levels were increased (Fig. 4C), confirming that the plasmids did effectively upregulate the HIF-1α protein under normoxia. Then we cotransfected overexpressing HIF-1α expression plasmids (pcDNA-HIF-1α) or control vector (pcDNA3.1) with pGL3-Fascin-promoter into 293T and MiaPaCa-2 cells. After incubation for 48 hours, cells were harvested for dual luciferase assay. The full-length fascin promoter activity increased in the presence of HIF-1α when compared with the control vector ($P < 0.05$; Fig. 4D). To investigate whether the HRE3 site was involved in the HIF-1α–induced expression, we mutate the HRE3 sequence from GCGTG to GCATG in the luciferase reporter (pGL3-Fascin-promoter-mutation). pGL3-Fascin-promoter-mutation reporter activities were no longer increased after HIF-1α overexpression (Fig. 4D). A VEGF luciferase reporter construct (pGL3-VEGF-promoter) was used as positive control for the HIF-1α response. Taken together, these data demonstrated that HIF-1α transactivated the fascin promoter through binding to the HRE3 region.

The correlation between HIF-1 and fascin expression in specimens of PDAC

To determine whether HIF-1 indeed regulates the expression of fascin in PDAC patients, we performed

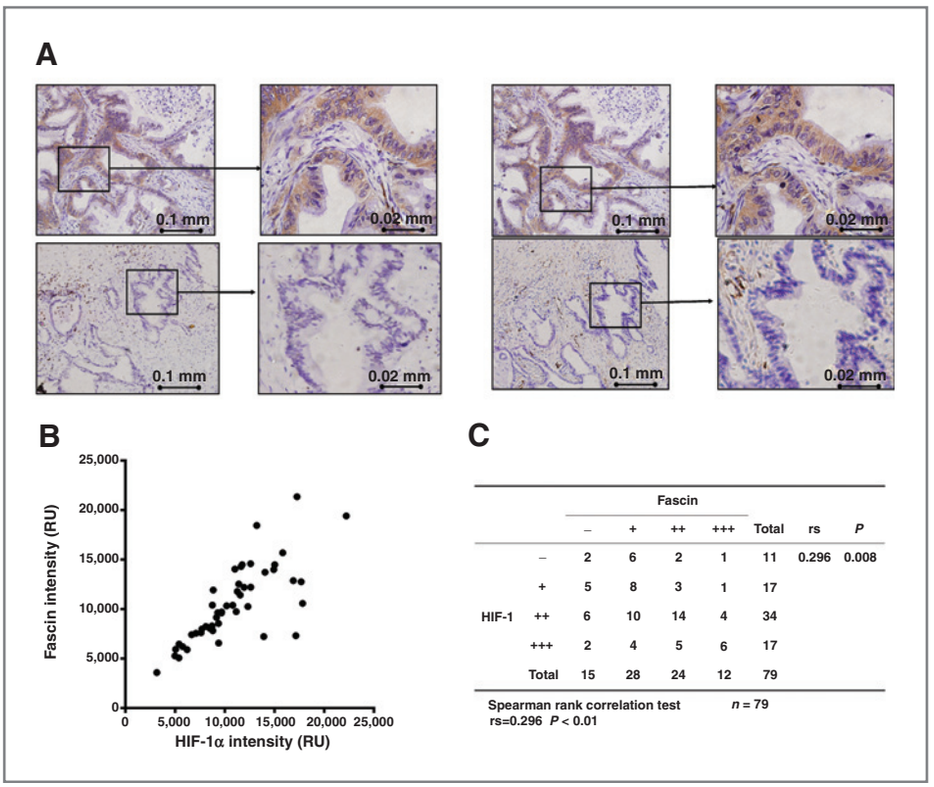

Figure 5. The correlation between HIF-1 and fascin expression in specimens of PDAC. A, immunohistochemical analysis of HIF-1α and fascin correlative expression in consecutive sections from human PDAC surgical samples. Left, fascin. Right, HIF-1α (magnification, ×200 or ×1,000). B, correlation between HIF-1α and fascin staining intensity in consecutive sections from PDAC surgical samples. HIF-1α and fascin staining intensity from 48 random regions in A were quantified using ImageJ software. C, statistical analysis of immunohistochemical results of HIF-1α and fascin expression in 79 human PDAC surgical samples. *P* values were calculated by the Spearman rank correlation test.

immunohistochemical staining to determine HIF-1α and fascin levels in a cohort of 79 PDAC specimens. As shown in Fig. 5A and B, fascin expression colocalizated with HIF-1α in consecutive sections of PDAC tissues. HIF-1α expression levels in PDAC tissues significantly correlated with the levels of fascin ($r = 0.77$, $P < 0.0001$), with HIF-1α high PDAC regions also overexpressing fascin (Fig. 5B). In addition, the relationship between HIF-1α and fascin expression showed that fascin expression was correlated with HIF-1α expression in PDAC surgical samples, suggesting that patients with hypoxic tumor microenvironment also overexpressed fascin (Fig. 5C; rs = 0.296, $P < 0.01$). Taken together, these data indicated that HIF-1 may play a critical role in fascin overexpression in PDAC.

Discussion

The fascin family consists of three isoforms, fascin-1, 2, and 3. Fascin-1, commonly referred to as fascin, is highly expressed in the spleen and brain, moderately in the lungs and placenta, weakly in the skeletal muscle, liver, and tonsil, and undetectably in the heart, kidney, pancreas, and bone marrow (28). Recent data from several groups have highlighted that fascin is upregulated in several human carcinomas, and fascin expression associates consistently with the most clinically aggressive tumors (29, 30). Our data indicated that fascin overexpression in PDAC tissue correlated with patient prognosis and advanced pathologic differentiation, suggesting that elevated protein levels of fascin promote PDAC progression. Our data further suggested that that overexpression of fascin in PDAC cells promotes invasion and increases MMP-2 expression. It is possible that high fascin expression in pancreatic cancer cells promotes invasion through inducing MMP-2.

In an effort to determine how fascin regulates MMP-2 expression, we investigated the upstream signaling pathways regulating MMP-2 expression. Our study showed that fascin overexpression increased the levels of phosphor-ERK. Pharmacologic inhibition of ERK blocked fascin-mediated MMP-2 expression, suggesting that fascin regulated MMP-2 expression through ERK signaling.

Fascin has been previously shown to be a substrate of the PKC-α (24, 31). Phosphorylation of the highly conserved Ser-39 site by PKC-α inhibits fascin actin-bundling activity and promotes the interaction between fascin and PKC-α (32, 33). Hashimoto and colleagues reported that the Ki67 index tended to be higher in fascin-positive areas compared with fascin-negative areas (34). Our data suggested that fascin overexpression increased PKC activity. It is possible that the binding of active PKC to fascin enhanced the phosphorylation of PKC substrates such as Raf-1 to promote cancer cell proliferation (35). Inhibition of PKC activity with bisindolymalei mide I or siRNA abrogated the fascin-mediated phosphorylation of ERK, suggesting that fascin activates ERK through PKC, which in turn results in MMP-2 expression and cell invasiveness.

Previous studies suggested that many factors regulated fascin expression in cancer cells, such as miR-145, EGF

receptor, and TGF-β (36–39). Our data revealed that hypoxia in the PDAC microenvironment upregulate fascin expression through HIF-1. We identified four HREs, in the promoter region of fascin gene. By chromatin immunoprecipitation and luciferase analysis, we provided evidence showing that HIF-1 bound to the third HRE region on the fascin promoter to upregulate transcription. Our results showed that HIF-1 is critical for fascin overexpression in PDAC. In addition, we showed that fascin overexpression could rescue HIF-1α silencing-induced decreased invasion of cells. These results suggested that HIF-1α promotes PDAC cell invasion through upregulating fascin expression.

In summary, our study showed that hypoxic microenvironment promotes PDAC invasion and metastasis through upregulating the expression levels of fascin. Hypoxia induces fascin overexpression through HIF-1–mediated transactivation of fascin promoter. Our data further suggested that fascin overexpression increased MMP-2 expression through PKC-ERK signaling, which may at least partially account for the elevated invasiveness in fascin-overexpressing PDAC cells. It is also worth noting that fascin has been implicated in invadopodium formation in melanoma and breast cancer (39–41). It remains to be determined whether fascin-mediated invadopodia formation is involved in PDAC invasion and metastasis. Several small-molecule inhibitors targeting fascin have been recently reported (42). Given the critical roles of fascin in PDAC invasion and metastasis, these compounds might be useful in the prevention and treatment of PDAC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: X. Zhao, S. Gao, H. Ren, S. Yang, J. Hao
Development of methodology: X. Zhao, S. Gao
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X. Zhao, H. Ren, W. Sun
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X. Zhao, S. Gao, H. Ren, W. Sun, J. Sun, S. Yang
Writing, review, and/or revision of the manuscript: X. Zhao, S. Gao, H. Ren, S. Yang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Gao, H. Ren, H. Zhang
Study supervision: H. Ren

Grant Support

This work was supported by grants from the National Natural Science Foundation of China (grant nos. 81302082, 81272685, 81172355, and 31301151), the Doctoral Fund of Ministry of Education of China (grant no. 20101202110002), the Key Program of Natural Science Foundation of Tianjin (grant nos. 09JCZDJC17100 and 11JCZDJC18400), major anticancer Technologies R&D Program of Tianjin (grant no. 12ZCDZSY16700), and a project of the Tianjin Educational Commission (grant no. 20100123).

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Received October 22, 2013; revised January 31, 2014; accepted February 21, 2014; published OnlineFirst March 5, 2014.

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