ARTICLE
Journal of Cellular Biochemistry 114:2603–2611 (2013)

Journal of Cellular Biochemistry

The CX3CL1/CX3CR1 Reprograms Glucose Metabolism Through HIF-1 Pathway in Pancreatic Adenocarcinoma

He Ren,† Tiansuo Zhao,† Junwei Sun,† Xiuchao Wang, Jingcheng Liu, Song Gao, Ming Yu, and Jihui Hao*

Key Laboratory of Cancer Prevention and Therapy, Department of Pancreatic Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin, 300060, China

ABSTRACT

One of the hallmarks of cancer is revised glucose metabolism that promotes cell survival and proliferation. In pancreatic cancer, the regulatory mechanism of glucose metabolism remains to be elucidated. In this study, we found that CX3CR1 is expressed in pancreatic cancer cells lines. Exogenous or transfected CX3CL1 increased glucose uptake and lactate secretion. CX3CL1 stimulated HIF-1 expression through PI3K/Akt and MAPK pathways. Furthermore, knockdown of HIF-1 blocked CX3CL1-modified glucose metabolism in pancreatic adenocarcinoma cells. In conclusion, the CX3CL1/CX3CR1 reprograms glucose metabolism through HIF-1 pathway in pancreatic cancer cells. J. Cell. Biochem. 114: 2603–2611, 2013.

KEY WORDS: CX3CL1; GLUCOSE METABOLISM; HYPOXIA-INDUCIBLE FACTOR-1; PANCREATIC ADENOCARCINOMA

Chemokines are a family of small molecular signaling factors involved in cell trafficking and adhesion. Chemokines bind to and activate a family of chemokine receptors (G-protein coupled receptors GPCRs), then triggering the downstream signal transduction pathways [Ransohoff, 2009]. To date, about 50 chemokines and 20 chemokine receptors have been identified and grouped into four families (CXC, CX3C, CC, and C), according to the positions of four cysteine residues [Sun et al., 2010]. Growing evidence has indicated the role of chemokines in tumor progression and metastasis. Tumor microenvironment consists of multiple cell types including mesenchymal, immune, and epithelial cancer cells. Chemokines are secreted by these cells and mediate the interaction among them.

CX3CL1 is the single cytokine of CX3C group of chemokines, also termed Fractalkine or Neurotactin. CX3CL1 exerts its biological function through binding to its specific receptor, CX3CR1 [Imai et al., 1997]. Recently, the CX3CL1/CX3CR1 axis is detected in many tumors and exerts multiple effects. In prostate cancer, the CX3CL1/CX3CR1 mediates the metastasis of cancer cells toward bone marrow [Shulby et al., 2004]; In ovarian cancer, CX3CL1 stimulates the proliferation of CX3CR-positive epithelial malignant cells [Gaudin et al., 2011]. As to pancreatic cancer, the CX3CR1 receptor mediates chemotatic migration toward neural cells expressing CX3CL1. Immunohistochemical analysis shows that CX3CL1 and CX3CR1 are overexpressed in cancer tissues compared with normal acinar and ductal cells of the peritumoral areas [Xu et al., 2012]. Furthermore, CX3CL1 overexpression is identified as an independent negative prognostic factor in overall survival [Marchesi et al., 2008], suggesting that the CX3CL1/CX3CR1 axis may play an important role in the pathogenesis of pancreatic cancer.

Tumor cells represent important alterations in energy metabolism in order to maintain uncontrolled cell growth and accelerate invasive properties [Vander Heiden et al., 2009]. Normal cells utilize glucose to generate energy through glycolysis in the cytosol and Krebs cycle in the mitochondria under aerobic conditions [Vander Heiden et al., 2009]. However, cancer cells reprogram glucose metabolism by facilitating the flux of glucose through glycolysis, while limiting the flux of glucose through Krebs cycle in mitochondria [Warburg, 1956]. These alterations in the flux of glucose under aerobic conditions have been termed aerobic glycolysis [Koppenol et al., 2011]. Such alterations in energy metabolism significantly

The authors have no conflict of interest.

†These authors contributed equally to this work.

Grant sponsor: National Natural Science Foundation of China; Grant numbers: 81272685, 81172355, 30973490, 30900596, 30901448; Grant sponsor: The key Program of Natural Science Foundation of Tianjin; Grant numbers: 10JCZDJC20200, 11JCZDJC18400.

*Correspondence to: Jihui Hao, Department of Pancreatic Cancer, Tianjin Cancer Hospital, Tianjin 300060, China. E-mail: jihuihao@yahoo.com

Manuscript Received: 6 June 2012; Manuscript Accepted: 4 June 2013

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 16 July 2013

DOI 10.1002/jcb.24608 • © 2013 Wiley Periodicals, Inc.

reduce ATP production but facilitate rapid synthesis of metabolites, which generate building blocks to support rapid cell proliferation [Deberardinis et al., 2008]. Decreased energy production during aerobic glycolysis is maintained by rapid glucose uptake [Koukourakis et al., 2006].

Pancreatic adenocarcinoma cells also demonstrate increased uptake of glucose [Tanimoto et al., 2010]. HIF-1 transactivates a series of gene regulating glucose uptake and metabolism such as GLUT-1 and PDK-1 [Semenza, 2011]. As a result, overexpression of HIF-1 increases glycolysis and inhibits the glucose into the tricarboxylic acid (TCA) cycle. Previous studies have shown that HIF-1 is constitutively expressed in pancreatic cancer [Wang et al., 2011], suggesting that hypoxia-independent mechanisms exist in the stabilization of HIF-1. Typically, HIF-1 is involved in the glucose metabolism of pancreatic cancer [Chen et al., 2003]. Knockdown of HIF-1 reduces the expression of GLUT-1 and aldolase, two key enzymes for glycolysis and then inhibits the glucose uptake in pancreatic cancer tissues, thus suppressing tumorigenicity. Ryu et al. (2008) reported that CX3CR1 was detected in cultured human aortic endothelial cells (HAECs). In response to CX3CL1 stimuli, HIF-1 protein expression was elevated and activated the downstream gene (VEGF-A and VEGF-C), thus mediating angiogenesis. In preliminary studies, we also found that CX3CL1 could activate hypoxia inducible factor (HIF)-1 in pancreatic cancer cells. Since HIF-1 is downstream of CX3CL1 and involved in the glycolysis of pancreatic cancer, we postulate that CX3CL1/CX3CR1 signaling pathway may regulate the glucose metabolism through HIF-1 in pancreatic adenocarcinoma.

In this study, we aim to investigate the role of CX3CL1/CX3CR1 in glucose metabolism of pancreatic cancer and the underlying mechanism, thus further understanding the relationship between chemokines and cancer metabolism.

MATERIALS AND METHODS

CELL CULTURE AND TREATMENTS

Human pancreatic adenocarcinoma cells (CFPAC, 8988, MiaPaca2, and Panc-1) and HEK293T cells were obtained from the American Type Culture Collection. For treatment, 5×10^5 cells in 2 ml of culture medium were seeded in each well of a 6-well culture plates. After the cells reached confluence, the medium was replaced by Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 0.2% bovine serum albumin (BSA) (Sigma Chemicals). After cells were starved overnight, the medium was removed and replaced by the same medium containing recombinant human CX3CL1 (Peprotech, Rocky Hill, NJ) at various concentrations. Glucose and lactate were measured in three wells at 24 h after cells were treated with CX3CL1. The concentration of glucose and lactate in the supernatant were measured using the Glucose Assay Kit (JianChen, NanJing, China) and the Lactate Assay Kit (JianChen, NanJing, China), respectively. Each experiment was repeated three times.

Cells were plated at a density of 5×10^5 cells/well in 6-well plates containing complete medium. When the cells were 80% confluent, cells were serum-starved overnight. Subsequently, cells were treated with (i) LY294002 (Cell Signaling Technology) at 20 μmol/L to inhibit PI3K pathway, (ii) PD98059 (Cell Signaling Technology) at 20 μmol/L to inhibit MAPK activity. Then human CX3CL1 (Peprotech) at 200 ng/ml was added for another time and the treated cells were lysed for Western blotting.

siRNA DUPLEXES, PLASMID CONSTRUCTS, AND TRANSIENT TRANSFECTION

Small interfering RNAs (siRNAs) against CX3CR1 and HIF-1α were designed and synthesized from Ribobio (Guangzhou, China). The target sequences for siCX3CR1 were #1: 5′-CCGCCAACTCCATGAACAA-3′, #2: 5′-CCGCAATGTGGAAACAAAT-3′, #3: 5′-CCAAGAGTGTCACCGACAT-3′, and siHIF-1α were #1: 5′-CTGATGACCAGCAACTTGA-3′, #2: 5′-CAATCAAGAAGTTGCATTA-3′, #3: 5′-GCACAGTTACAGTATTCCA-3′, respectively. The pEGFP-C1-CX3CR1 overexpression plasmids were designed and synthesized from Ribobio (Guangzhou, China).

For isolation of full-length cDNA fragments of CX3CL1, we employed a one step RT-PCR from RNA extracts of PANC-1 cells (a human pancreatic carcinoma cell line). An upstream primer, 5′-CCG CTC GAG CTA TGG CTC CGA TAT CTC TGT CGT GG, engineered with an *Xho*I restriction site (underlined), and a downstream primer, 5′-CGC GGA TCC TCA CAC GGG CAC CAG GAC ATA, engineered with a *BamH*I restriction site, were used for PCR to amplify the fragments of the CX3CL1 Coding sequence. This fragment was excised with *Xho*I and *BamH*I and subcloned into the plasmid pEGFP-C1 (Invitrogen) to make pEGFP-C1-CX3CL1.

For transfection, cells were plated at a density of 5×10^5 cells/well in 6-well plates containing complete medium. When the cells were 80% confluent, the siRNA duplexes, pEGFP-C1-CX3CR1 or pEGFP-C1-CX3CL1 overexpression plasmids were transfected into cells using lipofectamine-2000 (Invitrogen) for 48 h.

REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION

Cells were treated with human CX3CL1 and the total RNA was extracted from cells using the Tripure isolation Reagent (Invitrogen, Paisley, UK). Complementary DNA (cDNA) was synthesized using a first strand cDNA synthesis kit (TaKaRa, Dalian, China). Then, 1 μg sample of the cDNA was quantified by real-time PCR using primer pairs with SYBR Green PCR Master mix (TaKaRa, Dalian, China). The sense and the antisense primers were 5′-AGG TTA TGT GCC TGA AGT CG-3′ and 5′-TTT CTG AGT GCC TGC TGT-3′ (glucose transporter-1) (GLUT-1); 5′-CAG AGC AAG AGA GGC ATC C-3′ and 5′-CTG GGG TGT TGA AGG TCT C-3′ (β-actin). The quantity of PCR product of the genes was standardized using the quantity of β-actin product for each sample done in triplicate to obtain a relative level of gene expression.

WESTERN BLOTTING ANALYSIS

Whole-cell extracts were prepared by lysing cells with SDS lysis buffer supplemented with proteinase inhibitors cocktail (Sigma). Protein concentration was quantified using Pierce protein assay kit (Pierce). Protein lysates (20 μg) were separated by SDS-PAGE, and target proteins were detected by Western blotting with antibodies against HIF-1α, VEGF, β-actin (Santa Cruz Biotechnology), CX3CR1 (Abcam, ab8021), Akt, p-Ser473 Akt, MAPK, p44/42 MAPK (Cell Signaling Technology) and then treated with the appropriate HRP-conjugated secondary antibodies (Abmart, Shanghai, China). Protein

expression levels were detected using the reagents provided in the ECL Plus kit (Millipore corporation, Billerica).

INDIRECT FLOW CYTOMETRY

The cells were adjusted to 1×10^6 cells/ml in PBS. Each 100 μl aliquot of the pellet was incubated for 1 h at 37°C with 10 μl of primary antibodies against CX3CR1 (1:200). After centrifugation (200*g*, 10 min, 4°C) and wash with PBS, pellets was resuspended and performed with 10 μl of fluorescein isothiocyanate-labeled goat anti-rabbit IgG (Fab fragments) (Zhongshan Jinqiao Co., Ltd., Beijin, China) in the dark for 30 min at 4°C. Cells were washed with PBS for one time, fixed in 0.5 ml of 1% paraformaldehyde in PBS, and kept in the dark at 4°C until analyzed by flow cytometry (Beckmen Coulter FC50, California). Percentage of cells fluorescence was used to quantify CX3CR1 expression. Each analysis was performed in triple as described subsequently.

STATISTIC ANALYSIS

Statistical analysis was performed using SPSS 17.0 statistical software. All values are reported as mean ± standard deviation (SD). Statistical analysis was performed using Student's *t*-test and ANOVA test at the level of $P < 0.05$.

RESULTS

THE CX3CL1/CX3CR1 SIGNALING PATHWAY INCREASED GLUCOSE UPTAKE AND LACTATE SECRETION

CX3CL1 exerts its biological function through binding to its specific receptor, CX3CR1. Firstly, we detected the expression of CX3CR1 in pancreatic cancer cell lines. As shown in Figure 1A, Western blot showed that all four pancreatic cancer cell lines expressed CX3CR1 protein. In addition, flow cytometry analysis showed that CX3CR1 is expressed on the membrane of the pancreatic cancer, among which MiaPaCa-2 and Panc-1 cells have the highest levels of CX3CR1 (above 80% positive rate) and selected for the further analysis. As a negative control, the positive rate of CX3CR1 on HEK293T cells was below 3% (Fig. 1B).

To detect the role of CX3CL1 in glucose metabolism, MiaPaCa-2 and Panc-1 cells were subject to recombinant human CX3CL1 stimuli for 24 h. As shown in Figure 2A,B, CX3CL1 (50–200 ng/ml) dose-dependently increased glucose uptake in pancreatic cancer cells. In accordance with increased glucose uptake, we also found that the concentration of lactate, an end product of anaerobic glucose metabolism, was significantly higher in the CX3CL1-treated cells compared with controls (Fig. 2C,D). In addition, we transfected pEGFP-C1-CX3CL1 plasmids into cells and found that overexpression of CX3CL1 could also promote glucose uptake and lactate secretion (Fig. 2E,F).

To detect whether CX3CL1 affected glucose metabolism through its specific receptor, CX3CR1, we utilized three siRNAs targeting CX3CR1 in Panc-1 cells. Western blot showed that the three siRNAs reduced the expression of CX3CR1 in different levels (Fig. 3A). Flow cytometry analysis showed that 100 nM of siCX3CR1#2 or siCX3CR1#3 knocked down the expression of CX3CR1 on the cell membrane (Fig. 3B). Consistent with the reduced expression of CX3CR1 by siCX3CR1#2 and siCX3CR1#3, the CX3CL1-induced glucose uptake and lactate secretion were reversed dose-dependently both in Panc-1 (Fig. 3C,D) and MiaPaCa-2 cells (Fig. 3E,F).

THE CX3CL1/CX3CR1 INDUCED HIF-1α EXPRESSION THROUGH PI3K/Akt AND MAPK PATHWAYS

Recent evidence has suggested that CX3CL1 upregulated the expression of HIF-1α in HAECs [Ryu et al., 2008]. In this study, we observed rapid increase in the levels of HIF-1α protein in CX3CL1-treated pancreatic adenocarcinoma cells (Fig. 4A). Meanwhile, Akt and MAPK signaling pathways were activated. In addition, we pretreated pancreatic adenocarcinoma cells with the PI3K/Akt inhibitor LY294002 and the MAPK inhibitor PD98059. Then human CX3CL1 was added to the cells. We found that inhibition of p-Akt and

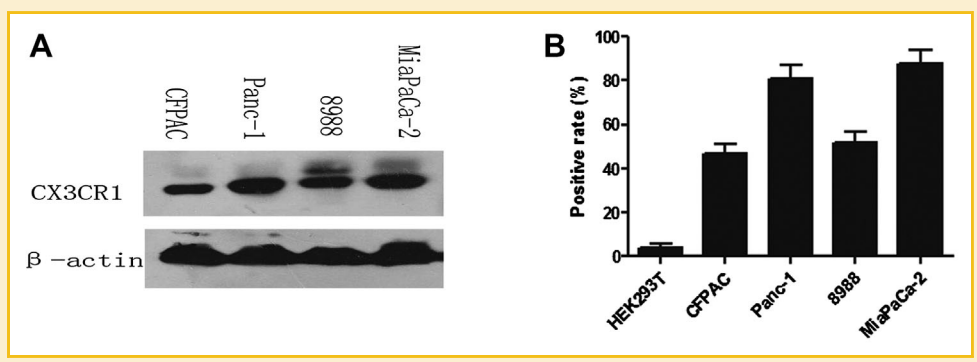

Fig. 1. Expression of CX3CR1 on pancreatic cancer cells. Four pancreatic cancer cell lines (CFPAC, Panc-1, 8988, MiaPaCa-2) were cultured in vitro and the basic expression of CX3CR1 was detected by (A) Western blot. Equal amounts of protein lysates were used for Western blot by antibodies against CX3CR1 and β- actin. And by (B) Indirect flow cytometry. Four pancreatic cancer cell lines were incubated with primary antibodies against CX3CR1 and then labeled with FITC-conjugated antibodies as described in Materials and Methods Section. HEK293T cells were introduced as a negative control. Results were expressed as the positive rate of CX3CR1 on the cell membrane. Columns, mean of three experimental determinations; Bars, standard deviation (SD).

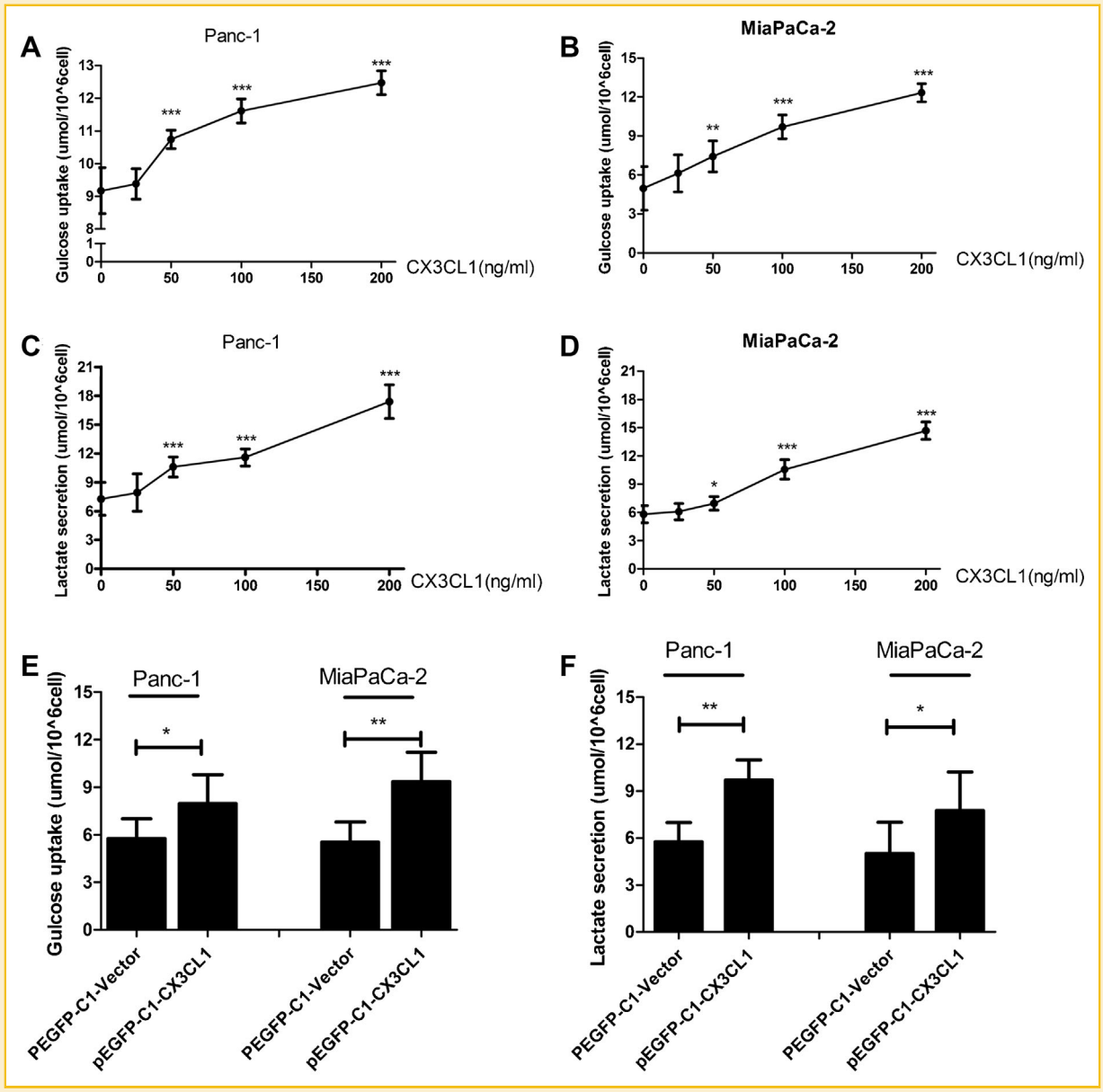

Fig. 2. CX3CL1 increased glucose uptake and lactate secretion. Panc-1 and MiaPaCa-2 cells were treated with different concentrations of recombinant human CX3CL1 (0–200 ng/ml) for 24 h and (A,B) Glucose uptake from culture medium was quantified and normalized for cell numbers. C,D: Lactate secretion in culture medium was quantified and normalized for cell numbers. E, F: Control vectors (PEGFP-C1-Vector) and overexpression vectors (PEGFP-C1-CX3CL1) (4 μg) were transfected into 3×10^5 cells using lipofactamine 2000 for 48 h and glucose uptake and lactate secretion were quantified and normalized for cell numbers. Data are presented as the mean $\pm$ SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the control. Columns, mean; Bars, SD.

p-MAPK prevented CX3CL1-induced HIF-1α expression in both the Panc-1 and MiaPaCa2 cell lines (Fig. 4B).

To verify the role of CX3CR1 in CX3CL1-induced HIF-1α expression, we transfected siCX3CR1#2 into pancreatic cancer cells and added CX3CL1 to 200 ng/ml. Consistent with the repression of CX3CR1, HIF-1α protein and mRNA were markedly decreased in pancreatic adenocarcinoma cells (Fig. 4C,D; $P < 0.01$). As a well-known target gene of HIF-1 [Liao and Johnson, 2007], VEGF mRNA and protein were concomitantly decreased, further reflecting the suppression of HIF-1.

REPROGRAMMING OF GLUCOSE METABOLISM BY CX3CL1 WAS DEPENDENT ON HIF-1α

Since HIF-1α is a transcription factor relating to the regulation of glucose metabolism, we postulate that CX3CL1 may regulate the glucose metabolism through HIF-1. To explore the function of HIF-1α, we knocked down HIF-1α gene in Panc-1 cells by three siRNAs. As shown in Figure 5A, the three siRNAs reduced the expression HIF-1α in different levels. Consistently, knockdown of HIF-1α by 100 nM of siHIF-1α#1 or siHIF-1α#2 dose-dependently counteracted CX3CL1-induced uptake of glucose and lactate secretion in Panc-1

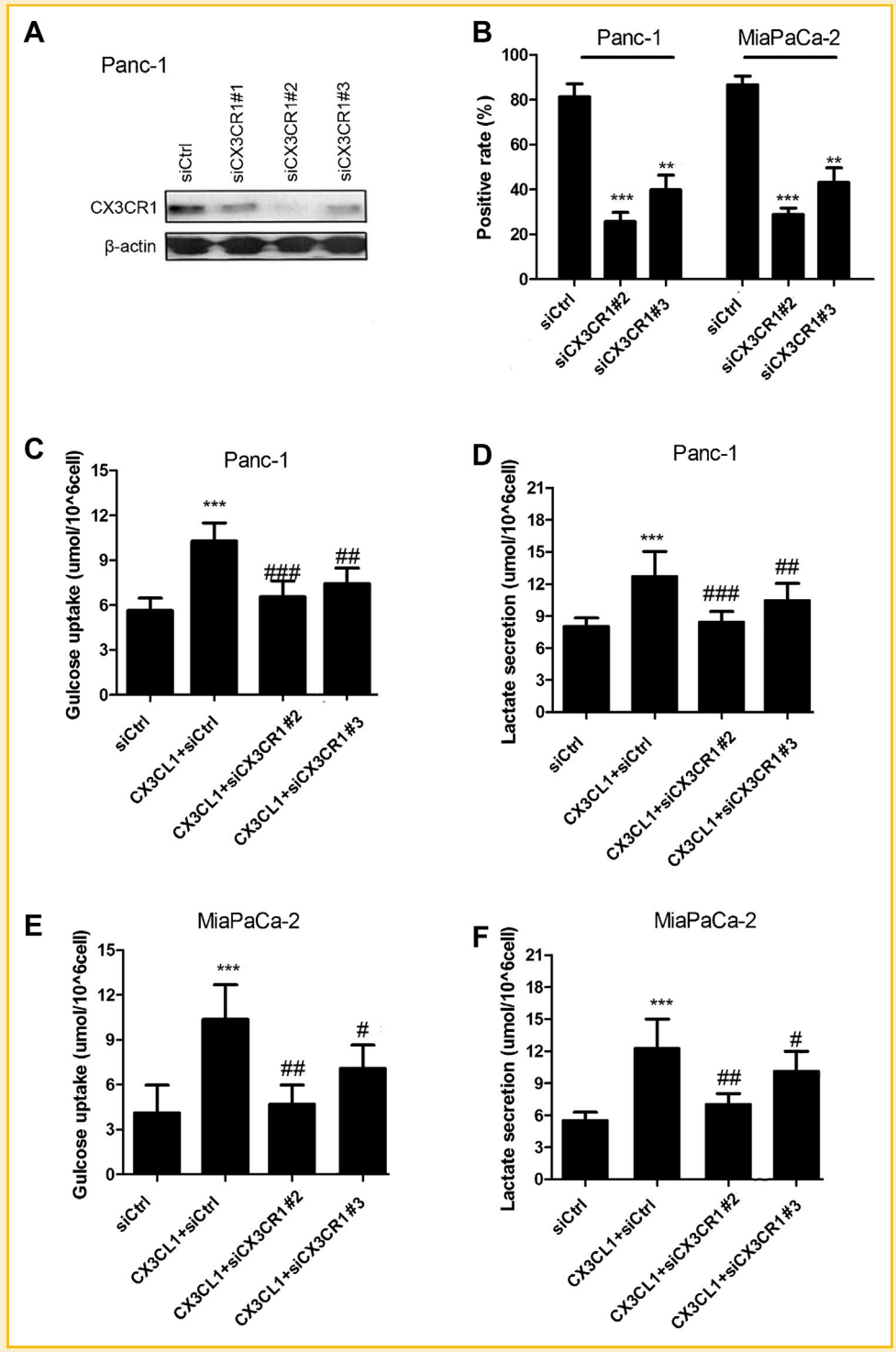

Fig. 3. CX3CL1 increased glucose metabolism via CX3CR1. A: Panc-1 cells were transfected with three specific siRNAs targeting CX3CR1 (siCX3CR1#1, siCX3CR1#2, and siCX3CR1#3) and nonsense siRNA (siCtrl) for 48 h and then cells were lysed for immunoblotting by anti-CX3CR1 or anti-β-actin antibodies. B: Panc-1 cells and MiaPaCa-2 cells were transfected with 100 nM of siCtrl, siCX3CR1#2 or siCX3CR1#3 for 48 h, and then the membrane positive rate of CX3CR1 protein was analyzed by indirect flow cytometry as described in Materials and Methods section. C,D: Panc-1 and (E,F) MiaPaCa-2 cells were transfected with 100 nM of siCtrl, siCX3CR1#2, or siCX3CR1#3 for 48 h and then incubated with CX3CL1 (200 ng/ml) for 24 h. Glucose uptake and lactate secretion of these cells were quantified and normalized for cell numbers. $^{***}P < 0.001$ versus the siCtrl group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus the CX3CL1 + siCtrl.

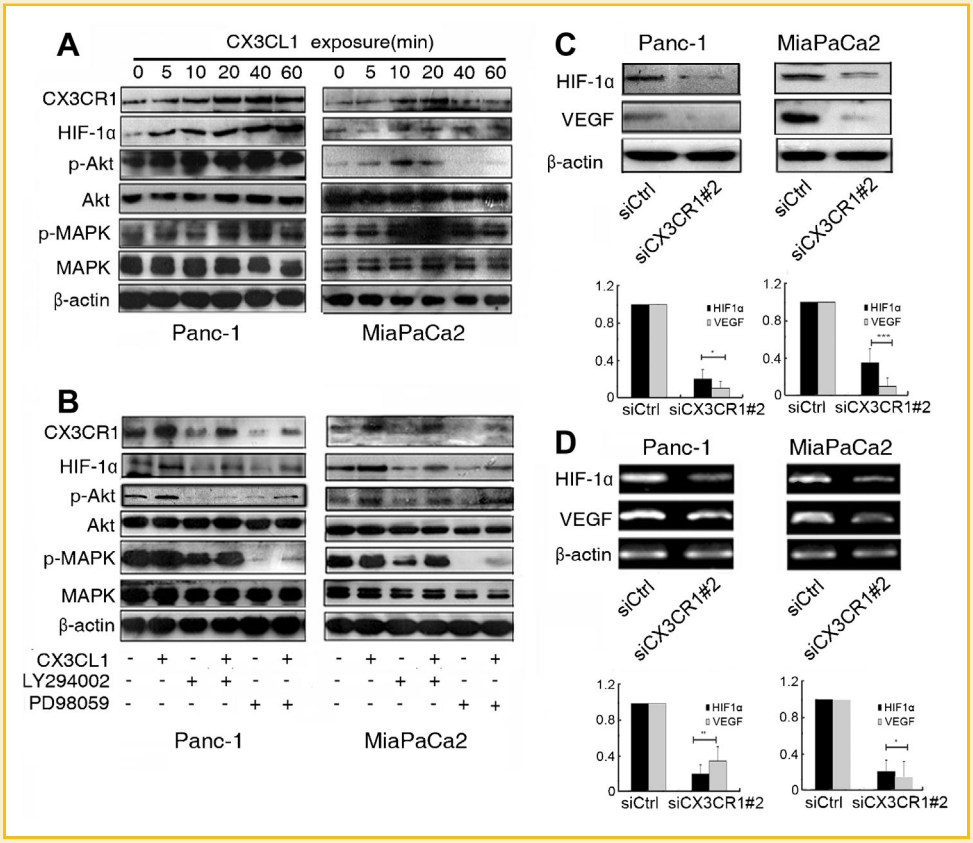

Fig. 4. CX3CL1 induced HIF-1α expression through PI3K/Akt and MAPK pathways. A: Panc-1 (left) and MiaPaCa2 (right) cells were treated with CX3CL1 (200 ng/ml) at different times and lysed for Western blotting. B: Panc-1 (left) and MiaPaCa2 (right) cells were pretreated with PD98059 (20 μM) and LY294002 (20 μM) for 2 h in free-serum medium. Then 200 ng/ml CX3CL1 was added for 20 min. Total cell lysates (20 μg) were examined by Western blotting. C: Panc-1 cells were transfected with siCX3CR1#2 (100 nM) for 48 h and then subject to CX3CL1 (200 ng) for 20 min. Total protein extracts were lysed for immunoblotting by anti-HIF-1, anti-VEGF or anti-β-actin antibodies (D) cells were lysed for RNA extraction and analyzed for the mRNA expression of HIF-1, VEGF or β-actin by real time quantitative PCR. Results were expressed compared to the control group. Column, mean of three independent experiments; Bars, SD.

cells (Fig. 5B,C) and MiaPaCa-2 (Fig. 5D,E). GLUT-1 is a target gene of HIF-1 involved in the glucose metabolism. We demonstrated that CX3CL1 activated the transcription of GLUT-1 and this effect was diminished following knockdown of HIF-1α (Fig. 5F).

DISCUSSION

Pancreatic cancer is a malignant disease with an extremely poor prognosis and the cellular mechanism is still unknown. Previous data support inflammation as a key mediator of pancreatic cancer development [Farrow and Evers, 2002]. CX3CL1, a chemokine that signals functions through a sole specific receptor (CX3CR1), has received considerable interest in relation to the pathogenesis of cancer. The expression of CX3CL1 could be enhanced by inflammatory stimuli, that is, TNF-α, interferon (IFN)-γ, and lipopolysaccharide [White et al., 2010]. Recent studies showed that CX3CL1-CX3CR1 axis was a relevant and independent risk factor to influence the prognosis of pancreatic adenocarcinoma patients [Marchesi et al., 2008] but the molecular mechanism remains to be defined.

Tumor cells reprogram the energy metabolism in order to maintain cell growth and accelerate glycolysis. In our study, we found that CX3CR1 is universally expressed on the membrane of multiple cell lines and exogenous CX3CL1 enhanced glucose uptake and lactate secretion, suggesting that CX3CL1 promotes glycolysis in pancreatic cancer. Recent evidence indicates that CX3CL1 is also secreted from pancreatic cancer cells [Xu et al., 2012]. To detect the autocrine effect of CX3CL1, we transfected CX3CL1 expression plasmids into cells and found that overexpression of CX3CL1 could also increase glucose uptake and lactate secretion. Through siRNA technology, we showed that CX3CL1 mediated glucose metabolism through CX3CR1 in pancreatic adenocarcinoma cells.

HIF-1 has been identified as a key factor to reprogram glucose metabolism. Previous studies showed that HIF-1 was constitutively expressed in pancreatic cancer. In addition to hypoxia, activation of PI3K and MAPK pathways (as a result of oncogene mutation of cytokine signaling) increases HIF-1α expression in cancer cells [Laughner et al., 2001]. We verified that CX3CL1 enhanced the expression of HIF-1α in pancreatic adenocarcinoma cells. Further studies showed that CX3CL1 influenced the expression of HIF-1α by MAPK and PI3K/Akt pathways. Interestingly, we found that CX3CR1

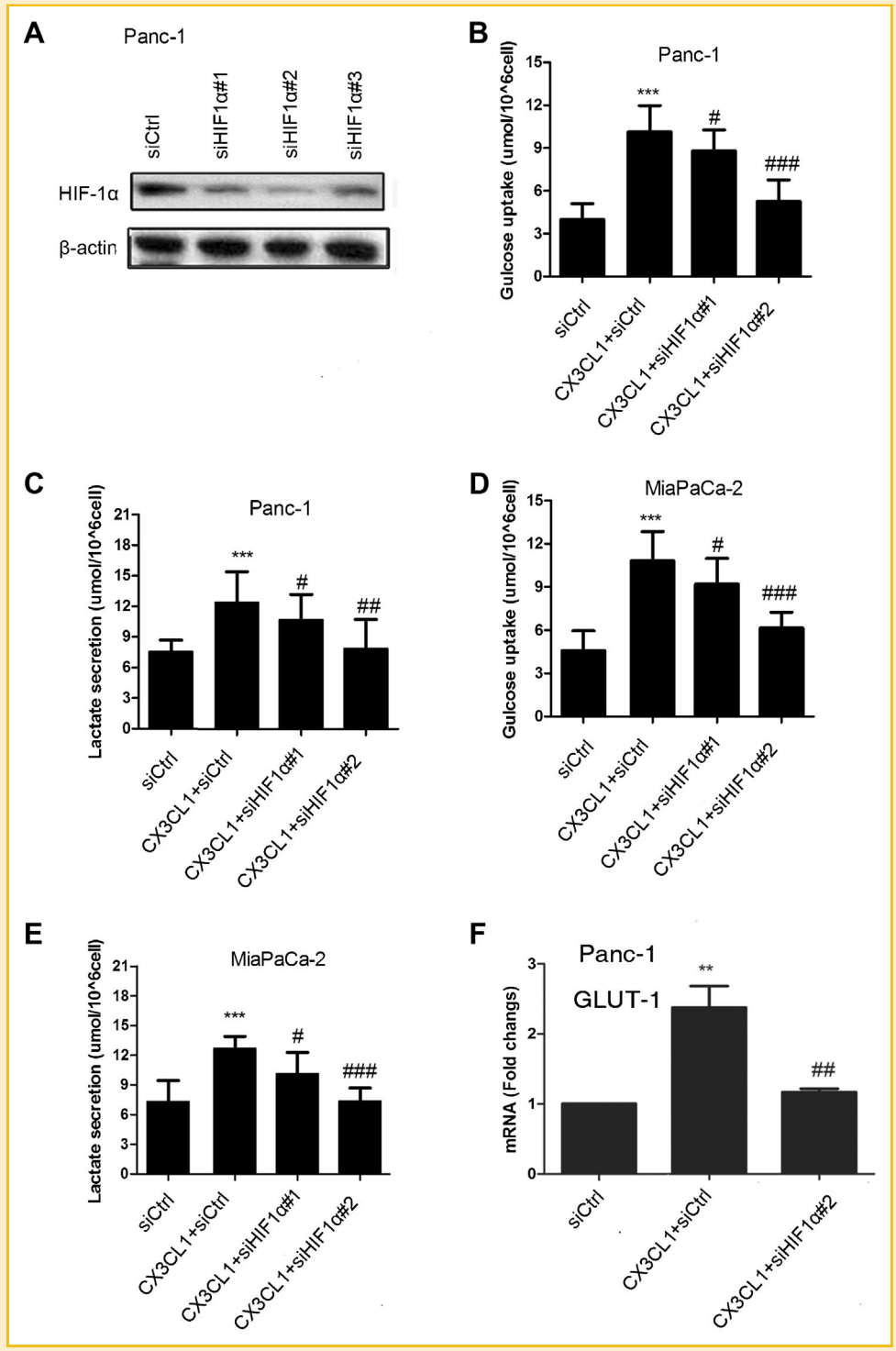

Fig. 5. Reprogramming of glucose metabolism by CX3CL1 was dependent on HIF-1α. A: Panc-1 cells were transfected with three specific siRNAs targeting HIF-1α (siHIF1α#1, siHIF1α#2, and siHIF1α#3) and nonsense siRNA (siCtrl) for 48 h and then cells were lysed for immunoblotting by anti-CX3CR1 or anti-β-actin antibodies. B,C: Panc-1 and (D,E) MiaPaCa-2 cells were transfected with 100 nM of siCtrl, siHIF-1α#1 or siHIF-1α#2 for 48 h and then incubated with CX3CL1 (200 ng/ml) for 24 h. Glucose uptake and lactate secretion of these cells were quantified and normalized for cell numbers. F: Then RNA was extracted from Panc-1 cells and GLUT-1 mRNA expression was quantified by real time quantitative RT-PCR. Results were expressed as fold induction relative to the control group, after normalization for the β-actin expression. $^{**}P < 0.01$ and $^{***}P < 0.001$ versus the siCtrl group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus the CX3CL1 + siCtrl group. Column, mean of three independent experiments; Bars, SD.

protein was increased rapidly after stimulation by CX3CL1 for 5–10 min. Although we recently confirmed that HIF-1α directly transactivated CX3CR1 [Zhao et al., 2012], the rapid increase of CX3CR1 could not be explained by the secondary response to HIF-1α up-regulation. It is reasonable that CX3CL1 may directly increase the stability of CX3CR1, thus resulting in a rapid accumulation of CX3CR1 protein. Up to now, the mechanism of CX3CR1 degradation is unclear. CX3CR1 is a member of G protein coupled receptors (GPCRs), most of which are posttranslational modified by ubiquitin that functions as a sorting signal for lysosomal degradation [Dores and Trejo, 2012]. For instance, CXCR4, a typical chemokine receptor in the family of GPCRs, was ubiquitinated at lysine residues after activated by its ligand and then degraded in lysosomes [Marchese and Benovic, 2001]. Further studies may focus on the role of ubiquitination in the degradation of CX3CR1 and of CX3CL1 binding in this process. Previous studies have formed a dogma that agonist stimulation initiates down-regulation of GPCRs [Tsao and von Zastrow, 2000]. In contrast, our findings suggest that some ligands, like CX3CL1, could stabilize the expression of the corresponding GPCRs and sensitize the downstream signaling pathways.

To further confirm the role of HIF-1 in CX3CL1-induced glycolysis, we used siRNA to down-regulate the expression of HIF-1 and found that glucose uptake and lactate secretion were concomitantly decreased. GLUT-1 is a key protein for glucose uptake and directly regulated by HIF-1 [Chen et al., 2003]. We demonstrated that CX3CL1 induced GLUT-1 expression and this effect was weakened by suppression of HIF-1, further supporting that HIF-1 mediates CX3CL1-induced metabolism.

Although it is well known that aerobic glycolysis is a key pathological feature of pancreatic adenocarcinoma, little is known about the mechanism compared with lymphogenous or hematogenous formation. In our opinion, CX3CL1/CX3CR1 signaling pathways upregulate the expression of HIF-1α by MAPK and PI3K/Akt pathways and then enhance the glucose uptake in pancreatic adenocarcinoma. Recent clinical trials suggest that the energy metabolism inhibitors (2-DOG, gossypol, delocalized lipophilic cations, and uncouplers) [Rodriguez-Enriquez et al., 2009] are effective and relatively safe regimens for the treatment of cancer. We suggest that the CX3CL1/CX3CR1/HIF-1 signaling pathway is an effective target to interfere with the glucose metabolism of pancreatic cancer. Pancreatic carcinogenesis has been shown to be closely related to inflammation. Our studies provide evidence that inflammation-related chemokines reprogram glucose metabolism and might be partly involved in the malignant transformation of pancreatic cells.

REFERENCES

Chen J, Zhao S, Nakada K, Kuge Y, Tamaki N, Okada F, Wang J, Shindo M, Higashino F, Takeda K, Asaka M, Katoh H, Sugiyama T, Hosokawa M, Kobayashi M. 2003. Dominant-negative hypoxia-inducible factor-1 alpha reduces tumorigenicity of pancreatic cancer cells through the suppression of glucose metabolism. Am J Pathol 162:1283–1291.

Deberardinis RJ, Sayed N, Ditsworth D, Thompson CB. 2008. Brick by brick: Metabolism and tumor cell growth. Curr Opin Genet Dev 18:54–61.

Dores MR, Trejo J. 2012. Ubiquitination of G protein-coupled receptors: Functional implications and drug discovery. Mol Pharmacol 82:563–570.

Farrow B, Evers BM. 2002. Inflammation and the development of pancreatic cancer. Surg Oncol 10:153–169.

Gaudin F, Nasreddine S, Donnadieu AC, Emilie D, Combadiere C, Prevot S, Machelon V, Balabanian K. 2011. Identification of the chemokine CX3CL1 as a new regulator of malignant cell proliferation in epithelial ovarian cancer. PLoS ONE 6:e21546.

Imai T, Hieshima K, Haskell C, Baba M, Nagira M, Nishimura M, Kakizaki M, Takagi S, Nomiyama H, Schall TJ, Yoshie O. 1997. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. Cell 91:521–530.

Koppenol WH, Bounds PL, Dang. CV. 2011. Otto Warburg's contributions to current concepts of cancer metabolism. Nat Rev Cancer 11:325–337.

Koukourakis MI, Giatromanolaki A, Harris AL, Sivridis E. 2006. Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: A metabolic survival role for tumor-associated stroma. Cancer Res 66:632–637.

Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. 2001. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: Novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 21:3995–4004.

Liao D, Johnson RS. 2007. Hypoxia: A key regulator of angiogenesis in cancer. Cancer Metastasis Rev 26:281–290.

Marchese A, Benovic JL. 2001. Agonist-promoted ubiquitination of the G protein-coupled receptor CXCR4 mediates lysosomal sorting. J Biol Chem 7:45509–45512.

Marchesi F, Piemonti L, Fedele G, Destro A, Roncalli M, Albarello L, Doglioni C, Anselmo A, Doni A, Bianchi P, Laghi L, Malesci A, Cervo L, Malosio M, Reni M, Zerbi A, Di Carlo V, Mantovani A, Allavena P. 2008. The chemokine receptor CX3CR1 is involved in the neural tropism and malignant behavior of pancreatic ductal adenocarcinoma. Cancer Res 68:9060–9069.

Ransohoff RM. 2009. Chemokines and chemokine receptors: Standing at the crossroads of immunobiology and neurobiology. Immunity 31:711–721.

Rodriguez-Enriquez S, Marin-Hernandez A, Gallardo-Perez JC, Carreno-Fuentes L, Moreno-Sanchez R. 2009. Targeting of cancer energy metabolism. Mol Nutr Food Res 53:29–48.

Ryu J, Lee CW, Hong KH, Shin JA, Lim SH, Park CS, Shim J, Nam KB, Choi KJ, Kim YH, Han. KH. 2008. Activation of fractalkine/CX3CR1 by vascular endothelial cells induces angiogenesis through VEGF-A/KDR and reverses hindlimb ischaemia. Cardiovasc Res 78:333–340.

Semenza GL. 2011. Regulation of metabolism by hypoxia-inducible factor 1. Cold Spring Harb Symp Quant Biol 76:347–353.

Shulby SA, Dolloff NG, Stearns ME, Meucci O, Fatatis A. 2004. CX3CR1-fractalkine expression regulates cellular mechanisms involved in adhesion, migration, and survival of human prostate cancer cells. Cancer Res 64: 4693–4698.

Sun X, Cheng G, Hao M, Zheng J, Zhou X, Zhang J, Taichman RS, Pienta KJ, Wang J. 2010. CXCL12/CXCR4/CXCR7 chemokine axis and cancer progression. Cancer Metastasis Rev 29:709–722.

Tanimoto K, Yoshikawa K, Obata T, Ikehira H, Shiraishi T, Watanabe K, Saga T, Mizoe J, Kamada T, Kato A, Miyazaki M. 2010. Role of glucose metabolism and cellularity for tumor malignancy evaluation using FDG-PET/CT and MRI. Nucl Med Commun 31:604–609.

Tsao P, von Zastrow M. 2000. Down-regulation of G protein-coupled receptors. Curr Opin Neurobiol 10:365–369.

Vander Heiden MG, Cantley LC, Thompson CB. 2009. Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science 324:1029–1033.

Wang X, Liu Y, Ren H, Yuan Z, Li S, Sheng J, Zhao T, Chen Y, Liu F, Wang F, Huang H, Hao J. 2011. Polymorphisms in the hypoxia-inducible factor-1alpha gene confer susceptibility to pancreatic cancer. Cancer Biol Ther 12:383–387.

Warburg O. 1956. On the origin of cancer cells. Science 123:309–314.

White GE, Tan TC, John AE, Whatling C, McPheat WL, Greaves DR. 2010. Fractalkine has anti-apoptotic and proliferative effects on human vascular smooth muscle cells via epidermal growth factor receptor signalling. Cardiovasc Res 85:825–835.

Xu X, Wang Y, Chen J, Ma H, Shao Z, Chen H, Jin. G. 2012. High expression of CX3CL1/CX3CR1 axis predicts a poor prognosis of pancreatic ductal adenocarcinoma. J Gastrointest Surg 16:1493–1498.

Zhao T, Gao S, Wang X, Liu J, Duan Y, Yuan Z, Sheng J, Li S, Wang F, Yu M, Ren H, Hao J. 2012. Hypoxia-inducible factor-1alpha regulates chemotactic migration of pancreatic ductal adenocarcinoma cells through directly transactivating the CX3CR1 gene. PLoS ONE 7: e43399.