Original Paper

CXCR4/Let-7a Axis Regulates Metastasis and Chemoresistance of Pancreatic Cancer Cells Through Targeting HMGA2

Guangfa Xiao^a Xitao Wang^b Yaqun Yu^c^aDepartment of General Surgery, Xiangya Hospital, Central South University, Changsha, ^bPancreatic Biliary Surgery, Xiangya Hospital, Changsha, ^cDepartment of Hepatobiliary and Pancreatic Surgery, The Affiliated Hospital of Guilin Medical College, Guilin, P.R.China**Key Words**

Pancreatic cancer • CXCR4 • Let-7a • HMGA2 • Metastasis and drug resistance

Abstract

Background/Aims: Pancreatic cancer cells (PCC) is one of the most risky cancers and gemcitabine (GEM) is the standard first-line drug for treating PCC. The PCC will develop drug resistance to GEM after a period of treatment. However, the detailed molecular mechanism of pathogenesis and drug resistance remains unresolved. **Methods:** we employed qRT-PCR and western blot to examine the expression level of CXCR4, let-7a and HMGA2. In addition, we used MTT assay to detect cell proliferation and transwell assay to measure migration and invasiveness. The expression level of epithelial marker E-cadherin and mesenchymal marker N-cadherin was detected by western blot. The apoptosis was determined using annexin V-FITC/PI apoptosis detection kit by flow cytometry. **Results:** we first proved that CXCR4 negatively regulated let-7a in PCC. Next, let-7a was confirmed to play crucial role in tumorigenesis, metastasis and drug resistance of pancreatic cancer cells Bxpc-3 and Panc-1 *in vitro* and *in vivo*. Finally, we identified HMGA2 as important downstream target of let-7a in PCC and overexpression of HMGA2 restores cell proliferation, metastasis and chemosensitivity of GEM inhibited by let-7a. **Conclusion:** Taken together, we show an important signaling pathway involved in pathogenesis and drug resistance of PCC, thereby providing deeper insight into molecular mechanism by which CXCR4/let-7a regulates tumorigenesis and drug resistance of PCC. These findings will help us develop new strategies for diagnosis and treatment of PCC.

© 2017 The Author(s)
Published by S. Karger AG, Basel**Introduction**

Pancreatic cancer, derived from normal pancreatic ductal epithelial cells, is one of the most risky cancer types [1-4]. Although the pathogenesis of pancreatic cancer cells has been well-studied for a long time, the exact molecular mechanism underlying drug resistance remains unclear. It has been well-established that cancers, including pancreatic cancer cells, are caused by accumulated genetic lesions [5]. Inactivation of TP53, SMAD4 and TGF β have been found by large-scale genome sequencing of pancreatic cancer cells samples [6, 7].

Dr. Guangfa Xiao,
Dr. Yaqun YuDepartment of General Surgery, Xiangya Hospital, Central South University, Changsha, (P.R.China); Department of Hepatobiliary and Pancreatic Surgery, The Affiliated Hospital of Guilin Medical College, No. 15 Lequn Road, Xiufeng District, Guilin 541002, Guangxi (China), E-Mail xiaoguangfa68@163.com/yuyaqun0222@sina.com

Despite increasing knowledge of understanding the reason for pathogenesis of pancreatic cancer cells, the effective anti-pancreatic cancer drug remains scarce. Currently, GEM is still thought as the standard drug to treat pancreatic cancer [3, 8], however, not all cases respond similarly to GEM due to primary and acquired drug resistance. Therefore, it is an important field of fully understanding the mechanism underlying the drug resistance.

CXCR4, the chemokine receptor, has been reported to play an critical role in tumorigenesis and metastasis of non small cell lung cancer (NSCLC) and pancreatic cancer [9, 10]. More importantly, the recent studies revealed that CXCR4 is correlated with different degree of chemosensitivity of a variety of cancer types, including chemosensitivity of pancreatic cancer to GEM [11-15]. Some researches has implicated some miRNAs (like miR-21, miR-200, miR-145) and p85 in the regulation of drug resistance of pancreatic cancer to GEM [1, 8, 16-18]. In 2010, Miao Zhong et al. demonstrated that overexpression of let-7a, hsa-miR-126 and hsa-miR-145 enhanced the sensitivity of non-small cell lung cancer (NSCLC) cells to gefitinib [19]. Consequently, overexpression of tumor-suppressive microRNAs might be a promising therapeutical strategies for cancers, including pancreatic cancer. Let-7a has been recognized as downstream target gene of CXCR4 responsible for chemosensitivity [12]. Nonetheless, the detailed mechanistics of how CXCR4 influences chemosensitivity of pancreatic cancer to GEM is not fully understood, which becomes an urgent issue to be addressed.

Let-7a, a member of microRNA let-7 family, has been linked to various cancer types, e.g., breast cancer, non small cell lung cancer (NSCLC), pancreatic cancer, colon cancer [19-22]. It has been reported that overexpression of let-7a could sensitize resistant breast cancer cells to epirubicin *in vitro* [22]. In some studies, let-7a has been shown to inversely correlate with well-known oncogenes, including HMGA2 [23], and positively regulate classical apoptosis-related gene CASP3 [24]. These findings revealed that let-7a is likely to modulate many downstream target genes responsible for drug resistance of pancreatic cancer.

Here, we investigate the role of signaling pathway CXCR4/let-7a/HMGA2 in the pathogenesis of pancreatic cancer and chemosensitivity to GEM. In this study, we found that CXCR4 negatively correlates with let-7a and regulates the expression level of let-7a in pancreatic cancer cell lines Panc-1 and BxPc3. Next, let-7a was proven to inhibit cell proliferation, metastasis and chemosensitivity of pancreatic cancer cells *in vitro* and *in vivo*. Finally, we identified HMGA2 as crucial downstream target for let-7a-induced phenotypes. To summarize, we highlight the indispensable role of CXCR4/let-7a/HMGA2 pathway in tumor-associated phenotypes and chemosensitivity of pancreatic cancer cells to GEM, which provides deeper understanding of pathogenesis and drug resistance of pancreatic cancer cell and helps develop more robust therapies for treating pancreatic cancer.

Materials and Methods

Cell culture

Human pancreatic ductal epithelial cells (HPDE6-C7) were cultured in keratinocyte serum-free (KSF) medium with 0.2 ng/ml EGF and 30 µg/ml bovine pituitary extract (Gibco). Pancreatic cancer cell lines Panc-1 and BxPc3 were grown in Dulbecco's Modified Eagle's Medium (DMEM) (hyclone) supplemented with 10% fetal bovine serum (FBS) (BI) and 1% penicillin (100 U/ml)/streptomycin (100 U/ml) (Invitrogen) at 37 °C under 5% CO₂.

Tissue samples

We collected 15 patients with pancreatic cancer and surgically cut tumor samples and normal tissue nearby during the period of 2015.2-2016.1. Tissue samples was immediately snap-frozen in liquid nitrogen and then stored at -196°C in liquid nitrogen until it was needed for RNA was extracted. This study was approved by the Ethics Committee of Xianya Hospital affiliated to Central South University.

RNA oligoribonucleotides, vector construction and transfection

let-7a mimic, control siRNA and siRNA against HMGA2 were synthesized by GenePharma (Shanghai, China). We cloned full length HMGA2 from human cDNA library and ligated into pCDNA3.1 vector. Cells

were transfected using Lipofectamine 2000 reagents (Thermo Scientific, Waltham, USA) according to manufacturer's instructions.

Western blot

Western blot analysis were performed as previously described [15]. Briefly, the cell pellet was resolved in 2×SDS loading buffer to make sample, 50µg of protein were loaded onto SDS-PAGE gel and subject to transfer to PVDF membrane, next, the membrane was blocked with 5% milk for 1h. primary and secondary antibodies were used to probe the target proteins, finally, the membrane were incubated with ECL and detected by bio-rad machine. The following antibodies were used: HMGA2 (Cell Signaling), rabbit polyclonal CXCR4 (Abcam), E-cadherin (BD Transduction), N-cadherin (BD Transduction) and β-actin (Sigma)

Real-time quantitative polymerase chain reaction (qRT-PCR)

Total RNA were extracted using Trizol as described earlier [14]. Then, 1µg RNA was subject to reverse transcription using the TAKARA PrimeScript Kit. For real time PCR, we performed this experiment using SYBR(Roche) as dye and detected the signal according to the standard protocol. The following primers were used:

CXCR4-F: AGCTGTTGGCTGAAAAGGTGGTCTATG,
CXCR4-R: GCGCTTCTGGTGGCCCTTGGAGTGTG;
let-7a-F: GATTCCTTTTCACCATTCACCCTGGATGTT,
let-7a-R: TTTCTATCAGACCGCCTGGATGCAGACTTT;
HMGA2-F: CTCAAAAGAAAGCAGAAGCCACTG,
HMGA2-R: TGAGCAGGCTTCTTCTGAACAAC;
U6 was used to normalize and the sequences:
U6-F: GGTGAGGTAGTAGTTGTATAGTT,
U6-R: CTCGCTTCGGCAGCACATATA.

Luciferase reporter assays

The HMGA2 wild-type and mutant 3'-UTR were created and cloned to the firefly luciferase-expressing vector psiCHECK-2. For the luciferase assay, cells were seeded triplicate in 12-well plates the day before transfection, and transfected with the HMGA2 WT or Mut 3'-UTR reporter vector; the cells were harvested and lysed, and the luciferase activities were assayed using the Dual-Luciferase Reporter System (Promega). Three independent experiments were performed.

Transwell assay

The cells were transfected with let-7a mimic or HMGA2. 24 hours later, the cells were starved in medium without serum for another 12 hr and then digested with trypsin, and then seeded on the top chambers of 24 well transwell culture inserts (promega). The medium supplemented with 20% serum was used as chemoattractant. After 24 hr, The cells were fixed for 10 min with 4% formalin.

Invasion assays were performed using the BD Bio-Coat Matrigel invasion assay system (BD Biosciences) according to the manufacturer's instructions [14]. The nontile or noninvasive cells were removed, while the lower side of the filter were stained with 0.005% crystal violet and counted.

Cell viability assay

This experiment was performed as described [15]. In brief, the cells were seeded in 96-well plates at a density of 5000 cells per well, after the treatment, cell growth was examined using MTT cell proliferation assay. The absorbance of cells were determined at OD490nm.

Flow cytometry

Cells (2×10⁵ cells) were digested by trypsin and then washed with PBS, next, the cells were subject to be washed with annexin V-binding buffer followed by incubation with 5 µl annexin V-FITC and 5 µl propidium iodide for 15 min at room temperature in the dark. Cells were then washed with binding buffer and subject to FACS. Bxpc-3 and Panc-1 were both grouped into wildtype, negative control and let-7a overexpression, and then treated with GEM at the concentration of 0.5µM and 100µM, respectively.

Xenograft experiment

A total of 48 nude mice between the ages of 5-6 week old were used per treatment for tumour xenograft experiments. Panc-1 cells were transfected with control or let-7a vector, 48h later, 1×10^6 cells were collected and injected subcutaneously per mouse. After 15 days, these mice were subject to PBS or 100mg/kg GEM injection every week until harvest. Tumour size and weight were measured. All mice were euthanized 35 days after subcutaneous injection. Tumours were then excised and photographed.

Statistical analysis

Each experiment was performed for three times, and all values were expressed as mean $\pm$ S.D., The differences between the groups were compared using two-tailed student's t-tests. * $p < 0.05$; ** $p < 0.01$.

Results

CXCR4 downregulates let-7a level in PCC

Based on the published studies, we hypothesized that CXCR4 regulates the let-7a expression level in pancreatic tumor cells. So, we first examined the let-7a gene expression level in a variety of clinical normal and pancreatic tumor tissues. In agreement with previous studies, the result showed that let-7a was decreased in tumor tissues compared to normal pancreatic tissue (Fig. 1A). In addition, we observed that let-7a inversely correlated with CXCR4

Fig. 1. CXCR4 downregulates let-7a level in PCC. (A) mRNA level of let-7a in normal and pancreatic tumor tissues. (B) Spearman correlation analysis of CXCR4 and let-7a expression level in pancreatic cancer cells. (C) qRT-PCR analysis of CXCR4 mRNA in HPDE6-C7, Bxpc-3 and Panc-1 cells. (D) Western blot analysis of CXCR4 protein level in HPDE6-C7, Bxpc-3, Panc-1 and MIAPACA-2. (E) qRT-PCR analysis of let-7a level in HPDE6-C7, Bxpc-3 and Panc-1. (F-G) qRT-PCR analysis of let-7a level in Bxpc-3 and Panc-1 treated with vehicle (negative control) and SDF-1a (100ng/ml) or AMD3100 (200nM). Values were presented as mean $\pm$ SD (N=3, * $P<0.05$ and ** $P<0.01$ by Student's t-test).

Fig. 2. Let-7a enhances cell proliferation, invasiveness and metastasis of PCC. (A) qRT-PCR analysis of let-7a in Bxpc-3 and Panc-1 transiently transfected with control miRNA or let-7a mimic. (B) Cell viability assay of Bxpc-3 and Panc-1 transiently transfected with control miRNA or let-7a mimic at the indicated time points. (C) Migration assay and statistical analysis of Bxpc-3 and Panc-1 transiently transfected with control miRNA or let-7a mimic. (D) Statistical analysis of invasive cell number of Bxpc-3 and Panc-1 transiently transfected with control miRNA or let-7a mimic. (E) Western blot analysis of E-cadherin, N-cadherin of Bxpc-3 and Panc-1 transiently transfected with control miRNA or let-7a mimic, β-actin serves as internal control. Values were presented as mean ± SD (N=3, *P<0.05 and **P<0.01 by Student's t-test).

expression by spearman analysis in pancreatic tumor tissues (Fig. 1B). To be a validation, we examined the CXCR4 mRNA and protein levels in various normal and pancreatic tumor cell lines (Fig. 1C and 1D). These data revealed that CXCR4 highly expressed in pancreatic tumor cells and lowly in normal pancreatic cell line (HPDE6-C7). Instead, the normal pancreatic cell line (HPDE6-C7) expressed more abundant let-7a (Fig. 1E). Whats more, we detected let-7a level in two pancreatic tumor cells Bxpc-3 and Panc-1 treated with 100ng/ml SDF-1a, cognate ligand of CXCR4 [13, 25]. The let-7a level decreased by ~50%, indicating the ability of CXCR4 to regulate let-7a level in pancreatic tumor cells (Fig. 1F). On the other hand, the let-7a mRNA level increased markedly after the cells treated with potent CXCR4 inhibitor, AMD3100 (200nM) (Fig. 1G). Taken together, we suggest that CXCR4 is likely to function in pathogenesis of PCC via let-7a.

Let-7a suppresses cell proliferation and metastasis of PCC

Although it has been reported that let-7a play important role in cancer cell proliferation, invasiveness, metastasis, we determined whether let-7a functions similarly in our study

Fig. 3. Let-7a increase chemosensitivity of PCC to GEM in vitro. (A) Cell viability (IC_{50}) assay of Bxpc-3 and Panc-1 treated for 72h at the indicated GEM concentrations. (B) Cell viability assay of Bxpc-3 and Panc-1 treated with 1.25 µM and 250 µM GEM, respectively, transiently transfected with control miRNA or let-7a mimic at the indicated time points. (C) Flow cytometry analysis of apoptosis in Bxpc-3 cells stained with Annexin V-FITC and PI. The cells were transiently transfected with control miRNA or let-7a mimic and treated with GEM. (D) Flow cytometry analysis of apoptosis in Panc-1 cells stained with Annexin V-FITC and PI. The cells were transiently transfected with control miRNA or let-7a mimic and treated with GEM. Values were presented as mean $\pm$ SD (N=3, *P<0.05 and **P<0.01 by Student's t-test).

systems. We measured the cell proliferation rate of mock or let-7a mimic-overexpressed Bxpc-3 and Panc-1 cell at different time points (Fig. 2A and 2B). Compared with negative control, overexpressed let-7a leads to significantly retarded cell growth (Fig. 2B). Next, we also examined whether let-7a inhibits migration and invasiveness of PCC. The results showed markedly attenuated ability of migration (Fig. 2C) and invasiveness (Fig. 2D) when PCC overexpressed let-7a. Finally, we explored whether let-7a overexpression suppressed metastasis of PCC through alteration of EMT-associated markers expression. The western blot analysis showed that epithelial marker E-cadherin protein level increased and mesenchymal

Fig. 4. Let-7a increase chemosensitivity of PCC to GEM *in vivo*. (A) qRT-PCR confirmation of let-7a-overexpressing Bxpc-3 stable cell lines. (B) Xenograft tumor assay of Bxpc-3 stable cell lines expressing let-7a treated with vehicle or GEM. (C) Statistical analysis of xenograft tumor volume. (D) qRT-PCR analysis of let-7a level of xenograft tumors. Values were presented as mean $\pm$ SD (N=3, *P<0.05 and **P<0.01 by Student's t-test).

marker N-cadherin substantially declined after overexpressing let-7a mimic in Bxpc-3 and Panc-1 cell (Fig. 2E). Altogether, these data suggested that let-7a played critical role in pancreatic tumor cells proliferation, invasiveness and metastasis.

Let-7a increases chemosensitivity of PCC to GEM *in vitro*

It has been reported that let-7a has the capacity to sensitize cancer cells to chemotherapy drugs, but the effect of let-7a on drug resistance of PCC has not been studied. Hence, we attempted to determine whether let-7a enhance chemosensitivity of pancreatic tumor cell Bxpc-3 and Panc-1 to GEM. The result showed that let-7a overexpression decreased the IC₅₀ value of Bxpc-3 from about 1.25 μ M to 1 μ M and that of Panc-1 from about 250 μ M to 200 μ M (Fig. 3A). The cell viability assay showed that let-7a overexpression led to growth retardation of these two cell lines in the presence of GEM (Fig. 3B). Besides, we performed apoptosis assay by flow cytometry, we observed that let-7a markedly increased the apoptosis rate induced by GEM from about 2.9% to 22.4%, 3.3% to 6.8% Bxpc-1 and Panc-1 cells, respectively, compared to wildtype control (Control) (Fig. 3C and 3D). Taken together, let-7a increases chemosensitivity of pancreatic cancer cells Bxpc-3 and Panc-1 to GEM.

Let-7a inhibits tumorigenesis and increases chemosensitivity of PCC to GEM *in vivo*

To further confirm that let-7a is responsible for increased sensitivity of pancreatic cancer cell to GEM, we undertook exnograf experiment of implanting let-7a-overexpressed cell into immunodeficient nude mice. We chose Bxpc-3 cell for the *in vivo* models. When the mice intracranially transplanted with Lv-let-7a that stably express let-7a, or control lentiviral empty vector (vector) (Fig. 4A). The result demonstrated that cells-overexpressing let-7a not only led to a significant inhibitory effect on tumorigenesis but also exerted more sensitivity to GEM (Fig. 4B and 4C). To verify the expression of let-7a in xenograft tumors, we extracted and determined the let-7a mRNA level of these tumors by qRT-PCR. As shown in Fig. 4D, there was a higher expression in LV-let-7a group. Taken together, let-7a played a crucial role in tumorigenesis and sensitivity of pancreatic cancer cell to GEM *in vivo*.

HMGA2 acts as downstream target of let-7a in PCC

To interrogate the molecular mechanism by which let-7a inhibits cell proliferation, invasiveness and chemoresistance of PCC, we needed to find out the downstream target of

Fig. 5. HMGA2 acts as downstream target of let-7a in PCC. (A) Bioinformatic prediction of let-7a target genes. (B) Luciferase reporter assay of reporter gene driven by wildtype(WT) or mutant(Mut) HMGA2 promoter region in Bxpc-3 and Panc-1 transiently transfected with control miRNA or let-7a mimic. (C) qRT-PCR analysis of HMGA2 mRNA level in Bxpc-3 and Panc-1 transiently transfected with control miRNA or let-7a mimic. (D) Westernblot analysis of HMGA2 protein level in Bxpc-3 and Panc-1 transiently transfected with control miRNA or let-7a mimic, β -actin serves as internal control. Values were presented as mean $\pm$ SD (N=3, *P<0.05 and **P<0.01 by Student's t-test).

Fig. 6. HMGA2 restores let-7a-suppressed cell viability and migration in PCC. (A) Westernblot analysis of Bxpc-3 and Panc-1 transfected with let-7a mimic alone or in combination with HMGA2. (B) Cell viability assay of Bxpc-3 and Panc-1 transiently transfected with let-7a mimic, HMGA2 or combination of let-7a mimic and HMGA2. (C) Transwell assay and cell number count of Bxpc-3 and Panc-1 transiently transfected with control miRNA, let-7a mimic alone or combination of let-7a mimic and HMGA2.

let-7a. According to bioinformatic analysis (<http://www.microrna.org>) and previous studies, we focused on HMGA2, a directly targeted gene by let-7a [26, 27] (Fig. 5A). To verify this finding, we conducted luciferase reporter assay. We observed that co-transfection of let-7a mimics and psi-HMGA2-wt significantly decreased the luciferase activity in Bxpc-3 and Panc-1 cells as compared with the negative control (NC). Moreover, let-7a mimics had no effect on

Fig. 7. HMGA2 rescues let-7a-suppressed cell proliferation and apoptosis in PCC. (A) Cell proliferation assay of Bxpc-3 and Panc-1, treated with 1.25 μM and 250 μM GEM, respectively, transiently transfected with control or siRNA against HMGA2. (B) Flow cytometry analysis of apoptosis in Bxpc-3 and Panc-1, treated with GEM, transiently transfected with control miRNA, let-7a mimic alone and combination of let-7a mimic and HMGA2. (C) Statistical analysis of apoptotic cell number in Fig. 7B. (D) Cell survival analysis of Bxpc-3 and Panc-1, treated with the indicated concentration of GEM, transiently transfected with control or siRNA against HMGA2. Values were presented as mean ± SD (N=3, *P<0.05 and **P<0.01 by Student's t-test).

the luciferase activity when co-transfected with psi-HMGA2-mut (Fig. 5B). In addition, we detected HMGA2 mRNA and protein level upon let-7a overexpression. The result showed that let-7a reduced HMGA2 protein level, while mRNA remained unchanged (Fig. 5C and 5D). Therefore, our results suggest that let-7a possibly decreased protein level of HMGA2 via binding 3'-UTR to impede translation of its mRNA.

Overexpression of HMGA2 reverses the inhibitory effect of let-7a on cell growth and metastasis

As HMGA2 was negatively regulated by let-7a, and a let-7a mimic can inhibitor cell growth and metastasis, we hypothesized that the overexpression of HMGA2 could lead to the same result. We examined the cell proliferation of Bxpc-3 and Panc-1 transfected with let-7a mimic alone or in combination with HMGA2 (Fig. 6A). Surprisingly, HMGA2 overexpression substantially rescued let-7a-induced cell proliferation retardation (Fig. 6B). Then, we used the transwell assay to detect the invasiveness capacity of PCC cells. Similarly, overexpressed HMGA2 was able to partially reconstitute the invasiveness capacity of Bxpc-3 and Panc-1 cell (Fig. 6C).

Overexpression of HMGA2 impairs the role of let-7a on increasing the sensitivity of PCC cells to gemcitabine

To verify that let-7a enhances sensitivity of PCC cells to GEM through negatively regulating HMGA2 expression, we first used the MTT assay to detect the cell proliferation of PCC cell in different GEM concentrations. The result shown that the regulatory ability of let-7a on

the sensitivity of PCC cell lines to GEM was reversed when HMGA2 was overexpressed (Fig. 7A). Additionally, overexpression of HMGA2 blocked apoptosis resulted from let-7a mimic overexpression (Fig. 7B, C). Finally, we investigated whether HMGA2 could antagonized the increased sensitivity of cells to GEM caused by let-7a. We knocked down HMGA2 using short interference RNA, resulting in decreased IC₅₀ value (Fig. 7D). To sum up, HMGA2 acted as downstream effector of let-7a to markedly antagonize alteration of cell chemosensitivity of pancreatic cancer cells caused by let-7a.

Discussion

Pancreatic cancer remains one of the most lethal malignancies, and insights into both personalized diagnosis and intervention of this disease are urgently needed. For patients with advanced pancreatic cancer, it is common to treat the patients with chemical drugs; however, the resulting drug resistance leads to decreased efficacy. In this study, we identified CXCR4/let-7a/HMGA2 pathway to regulate cell proliferation, metastasis and drug resistance of PCC. We first proved that CXCR4 inversely correlates with let-7a in PCC and CXCR4 negatively regulates let-7a level at tissue and cellular level. This finding prompted us to concentrate our attention on let-7a, a well-studied miRNA implicated in cancer cell tumorigenesis, metastasis and chemosensitivity. We next investigated the role of let-7a in PCC and confirmed let-7a functions similarly like in other types of cancer cells. Finally, we identified HMGA2 as downstream target of let-7a to modulate cell proliferation, metastasis and chemosensitivity to GEM. In sum, we report a whole pathway responsible for tumorigenesis, metastasis and chemosensitivity to GEM in pancreatic cancer cells.

CXCR4 is a chemokine receptor and activated by its ligand SDF-1a [25]. And CXCR4 has been reported to play oncogenic role in various types of cancers (including pancreatic cancer) and enhance drug resistance of PCC [9, 13, 14]. Meanwhile, it had report that CXCR4 was inversely correlate with let-7a in acute myeloid leukemia [12]. It has been recognized that let-7a play suppressive role in tumorigenesis and chemosensitivity of cancer cells [19, 20, 28]. However, the role of CXCR4/let-7a in tumorigenesis and drug resistance of PCC remains not well-studied. Thus, we interrogated the role of CXCR4/let-7a in proliferation, metastasis and drug resistance of pancreatic cancer cells *in vitro* and *in vivo*. Consistently, the results demonstrates that let-7a inhibits tumorigenesis and drug resistance of PCC.

Although let-7a has been well-established to play important role in cell proliferation, invasiveness, metastasis and drug resistance, the molecular mechanism by which let-7a functions is not fully known. Recently, let-7a seems to exert its function through regulation of HMGA2 expression in nasopharyngeal carcinoma and esophageal squamous cell carcinoma [29, 30]. By combination of bioinformatic prediction and experimental confirmation, we identified HMGA2 as downstream target of let-7a in PCC and effector in let-7a-induced attenuated cell proliferation, metastasis and drug resistance of PCC. However, to systematically identify downstream target of let-7a, we can utilize RNA-seq approach to search for all directly and indirectly regulated genes by let-7a responsible for tumorigenesis, metastasis and drug resistance of PCC.

In summary, we propose a signaling pathway CXCR4/let-7a/HMGA2 responsible for regulation of cell proliferation, metastasis and drug resistance of PCC. These findings will help provide a solid foundation for understanding the molecular basis of pathogenesis and drug resistance of pancreatic cancer cells and develop new strategies for prevention, diagnosis and treatment of PCC.

Disclosure Statement

There is no conflict of interest.

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