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miR-125a-3p is responsible for chemosensitivity in PDAC by inhibiting epithelial-mesenchymal transition via Fyn

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ABSTRACT

Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers and resistance to cytotoxic chemotherapy is the major cause of mortality in PDAC patients. miR-125a-3p was found to be down-regulated in PDAC cells; however, the function of miR-125a-3p in PDAC has been elusive. Here, we explored the role of miR-125a-3p in chemosensitivity in PDAC cells.

Methods: We used qRT-PCR to detect miR-125a-3p expression in two PDAC cell lines. And we measured cell viability and apoptosis by MTT assay and flow cytometry, respectively. Scratch wound healing assay and transwell invasion assay were used to test the effects of miR-125a-3p and Fyn on cell EMT process. In addition, we validated the interaction of miR-125a-3p and Fyn by dual luciferase reporter assay. qRT-PCR and western blot were used to detect the mRNA and protein expressions of E-cadhrein, N-cadhrein, Snail and Fyn.

Results: We found that miR-125a-3p was down-regulated in a time-dependent manner following treatment with gemcitabine in PDAC cells. Meanwhile, we found that overexpression of miR-125a-3p significantly increased chemosensitivity to gemcitabine and suppressed epithelial-mesenchymal transition (EMT) of PDAC cells. Mechanistically, miR-125a-3p directly targeted Fyn and decreased the expression of Fyn that functions to promote EMT process in PDAC. Furthermore, overexpression of Fyn could partially reverse the effects of miR-125a-3p on chemosensitivity to gemcitabine.

Conclusion: Our study is the first to show that miR-125a-3p is responsible for chemosensitivity in PDAC and could inhibit epithelial-mesenchymal transition by directly targeting Fyn. This provides a novel potential therapeutic strategy to overcome chemoresistance in PDAC.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies and the fourth most common cause of cancer-related deaths worldwide [1–3]. Owing to the difficulty of diagnosing the disease at early stages, most patients are diagnosed at an advanced stage when surgical resection is not feasible. Chemotherapy like gemcitabine is the main treatment for patients at advanced stages. However, less than 20% of PDAC patients are sensitive to chemotherapeutic agents. As a result, the prognosis of PDAC is very poor, with a five-year survival rate of less than 5% [3]. Given the fact that chemoresistance is the major impediment for treating PDAC, there is clear need to understand the underlying molecular mechanisms, which could help develop more effective pharmacological therapies.

The epithelial to mesenchymal transition (EMT) is a process during which epithelial cells lose their polarization and homotypic cell adhesion, and gain properties of mesenchymal cells through multiple biochemical changes [4,5]. It plays key roles in numerous developmental processes including mesoderm formation and neural tube formation, as well as in tumor invasion and metastasis [4,6–9]. Besides that EMT has been recognized as a key mechanism of acquiring drug resistance, cell migration and invasion properties in cancers. For example, in PDAC, pancreatic cancer cells with gemcitabine resistance display features of EMT with enhanced mobility, invasion and resistance to apoptosis [10–12]. However, the mechanisms and pathways that drive EMT programs during the progress of PDAC cells resistance remain largely unknown.

MicroRNAs (miRNAs) are a class of endogenous, small, and non-coding RNAs (19～25 nucleotides). They negatively regulate gene expressions by binding the 3′-untranslated region (3′ UTR) of target messenger RNAs (mRNAs), resulting in mRNA degradation or inhibition of translation [13]. miRNAs play important roles in regulating multiple cellular processes such as cell differentiation, proliferation, chemoresistance and metastasis [14,15]. In PDAC, multiple miRNAs like miR-

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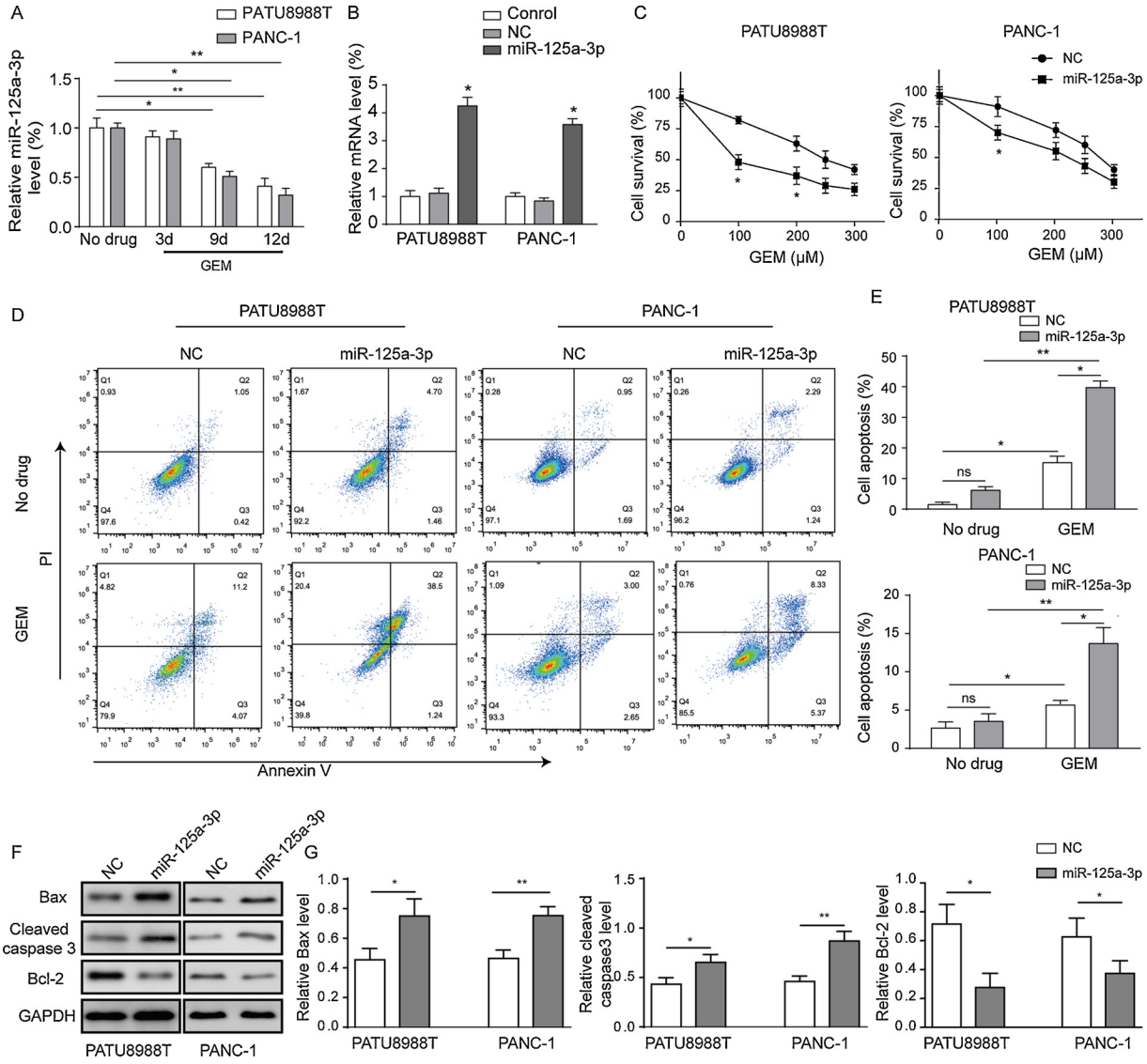

Fig. 1. miR-125a-3p suppresses PDAC chemoresistance to GEM in vitro. (A) qRT-PCR analysis of miR-125a-3p level in PATU8988 T cells and PANC-1 cells treated with GEM (50 μM) for various days. (B) miR-125a-3p level from control cells, cells transfected with miR-125a-3p mimics and negative control mimics (NC) was determined by qRT-PCR (*P < 0.05; **P < 0.01; n = 3). (C) Cell viability was measured using a MTT assay to calculate the IC_{50} values of gemcitabine (GEM) in cells transfected with miR-125a-3p mimics or NC, followed by various concentrations of GEM treatment. (D) Cell apoptosis was determined by Annexin-V-FITC/PI staining and flow cytometry analysis in cells transfected with NC and miR-125a-3p mimics with GEM or No-drug treatment (*P < 0.05; **P < 0.01; n = 3). (E) Quantification of cell apoptosis percentage. (*P < 0.05; **P < 0.01; n = 3). (F-G) Western blot analysis of Bax, Cleaved-caspase-3, Bcl-2 levels in cells transfected with NC and miR-125a-3p mimics with GEM treatment (*P < 0.05; **P < 0.01; n = 3).

155, miR-21, miR-181b have been observed dysregulated and are involved in the regulation of drug resistance. For example, miR-155 promotes gemcitabine resistance in PDAC by controlling exosome synthesis [16]; miR-21 is up-regulated in PDAC and enhances malignancy of pancreatic cancer cells [17]; miRNA-181b sensitizes PDAC cells to gemcitabine by targeting BCL-2 [18]. miR-125a-3p, a developmentally regulated miRNA, was previously shown to be down-regulated in PDAC [19]. Nevertheless, the exact functions of miR-125a-3p in PDAC and gemcitabine resistance are not yet understood. Previous studies suggest that miR-125a-3p is involved in the regulation of EMT and chemoresistance in prostate cancer cells [19–21]. We thus hypothesize that miR-125-3p regulates chemoresistance in PDAC through EMT process.

In this study, we fully investigated the role of miR-125a-3p in PDAC. We found that miR-125a-3p was down-regulated in PDAC cells in a time-dependent manner upon gemcitabine treatment. Moreover, overexpression of miR-125a-3p could enhance cell chemosensitivity to gemcitabine and suppressed EMT in PDAC cells. We also identified Fyn as the downstream target of miR-125a-3p and increasing Fyn expression reversed the effect of miR-125a-3p. Altogether, our data suggest that miR-125a-3p plays a key role in the regulation of PDAC chemoresistance, which might be a promising target for future therapy development.

2. Materials and methods

2.1. Cell culture and transfection

The PATU8988 T, PANC-1 human pancreatic cancer cell lines were purchased from Central South University (Changsha, China). All cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

miR-125a-3p mimics, negative control mimics (NC), Fyn shRNA (si-