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miR-210 regulates the interaction between pancreatic cancer cells and stellate cells

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ABSTRACT

There is accumulating evidence that pancreatic stellate cells (PSCs) promote the progression of pancreatic cancer. microRNAs (miRNAs) are small non-coding RNAs acting as negative regulators of gene expression at the post-transcriptional level. This study aimed to clarify the role of miRNAs in the interaction between PSCs and pancreatic cancer cells. Pancreatic cancer cells were mono-cultured or indirectly co-cultured with PSCs. miRNAs were prepared, and Agilent's miRNA microarray containing probes for 904 human miRNAs was used to identify differentially expressed miRNAs. miR-210 was identified as an upregulated miRNA by co-culture with PSCs. Conditioned media of PSCs activated ERK and Akt, but not hypoxia-inducible factor-1α pathway. PSCs-induced miR-210 upregulation was inhibited by inhibitors of ERK and PI3K/Akt pathways. Inhibition of miR-210 expression decreased migration, decreased the expression of vimentin and snai-1, and increased the membrane-associated expression of β-catenin in Panc-1 cells co-cultured with PSCs. In conclusion, our results suggest a novel role of miR-210 in the interaction between PSCs and pancreatic cancer cells.

1. Introduction

Pancreatic ductal adenocarcinoma is a highly malignant phenotype characterized by rapid progression, early metastasis, and a limited response to chemotherapy and radiotherapy [1–3]. The abundant desmoplastic/stromal reaction is a characteristic feature of pancreatic cancers [3,4]. It has been recognized that the cells responsible for the production of the desmoplastic reaction in pancreatic cancer are pancreatic stellate cells (PSCs) [4–10]. In normal pancreas, PSCs are quiescent and can be identified by the presence of vitamin A-containing lipid droplets in the cytoplasm. In response to pancreatic injury or inflammation, they are transformed ("activated") from their quiescent phenotype into myofibroblast-like cells, which express α-smooth muscle actin, actively proliferate, and produce extracellular matrix components such as type I collagen [4–10]. For more than a decade, evidence has been accumulating that activated PSCs play a pivotal role in the development of pancreatic fibrosis in chronic pancreatitis and pancreatic cancer [4–10]. microRNAs (miRNAs) are small, non-coding RNAs consisting of 17–25 nucleotides that regulate gene expression by binding loosely complementary sequences in the 3′-untranslated region of target mRNAs to repress translation or induce mRNA cleavage [11,12]. miRNAs regulate a variety of cell functions such as cell proliferation, development, apoptosis, differentiation, and carcinogenesis [11,12]. Although previous studies have clarified the roles of miRNAs in pancreatic cancer [13–17], no studies have addressed the role of miRNAs in the interactions between PSCs and pancreatic cancer. To address this issue, we compared the miRNA expression profiles between mono-cultured cancer cells and those co-cultured with PSCs. We here show that PSCs induce the expression of miR-210 in pancreatic cancer cells.

2. Materials and methods

2.1. Materials

Mouse anti-hypoxia-inducible factor (HIF)-1α antibody was purchased from Novus Biologicals (Littleton, CO). Mouse anti-vimentin and rabbit anti-Snai1 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse anti-β-catenin antibody was purchased from BD Biosciences (Franklin Lakes, NJ). Rabbit antibodies against ERK (total and phosphorylated at Tyr^{202}/Tyr^{204}) and Akt (total and phosphorylated at Ser^{473}) were purchased from Cell Signaling Technologies (Bevery, MA). Rabbit

Abbreviations: BrdU, 5-bromo-2′-deoxyuridine; DAPI, 4′, 6-diamidino-2-phenylinodole; EMT, epithelial-mesenchymal transition; HIF, hypoxia-inducible factor; miRNA, micro RNA; OD, optical density; PSCs, pancreatic stellate cells; PSC-CM, conditioned medium of hPSC21-S/T cells.

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anti-GAPDH antibody was purchased from R&D Systems (Minneapolis, MN). U0126, LY294002, and wortmannin were purchased from Merck Millipore (Billerica, MA). Other reagents were purchased from Sigma–Aldrich (St. Louis, MO) unless specifically described.

2.2. Cell culture

The human pancreatic cancer cell lines Panc-1 and MIAPaCa-2 were obtained from the American Type Culture Collection (Manassas, VA). The human pancreatic cancer cell line SUIT-2 was obtained from the Japanese Collection of Research Bioresources (Ibaraki, Osaka, Japan). Panc-1 and SUIT-2 cells were grown in RPMI1640 medium supplemented with 10% FBS, penicillin sodium, and streptomycin sulfate. MIAPaCa-2 cells were maintained in DMEM supplemented with 10% FBS, penicillin sodium, and streptomycin sulfate.

The immortalized human PSC line hPSC21-S/T was established by retrovirus-mediated gene transfer of simian virus 40 T antigen and human telomerase reverse transcriptase into the human PSCs isolated from the resected pancreas tissue of a patient undergoing operation for pancreatic cancer [18]. Cells were maintained in Ham's F-12/DMEM (1:1) supplemented with 10% FBS, penicillin sodium, and streptomycin sulfate. This study was approved by the Ethics Committee of Tohoku University School of Medicine.

The conditioned media of hPSC21-S/T cells was harvested following 72-h incubation, centrifuged at 3000 revolution/min for 10 min, filtered through 0.22-μm filters and concentrated 10-fold through 3000 molecular weight cut-off filters (Centriprep YM3; Merck Millipore) according to the manufacturer's instruction. The concentrated media was designated as PSC-CM.

2.3. Hypoxic treatment

Cells were usually incubated under normoxia (21% O_2, 5% CO_2, and 74% N_2). However, for some experiments, Panc-1 cells were incubated in a hypoxic incubator with 1% O_2, 5% CO_2, and 94% N_2. The oxygen level in the culture chambers was continuously monitored. Culture medium was preconditioned to the correct O_2 level in the hypoxic experiments.

2.4. Indirect co-culture of pancreatic cancer cells and PSCs

Pancreatic cancer cells (1×10^5 cells/well) were seeded in 6-well culture plates (BD Biosciences) in RPMI1640 medium supplemented with 10% FBS, penicillin sodium, and streptomycin sulfate. hPSC21-S/T cells (1×10^5 cells/culture insert) were seeded into culture inserts of 1.0 μm pore size (BD Biosciences) in Ham's F-12/DMEM (1:1) supplemented with 10% FBS, penicillin sodium, and streptomycin sulfate. Next day, the culture insets seeded with hPSC21-S/T cells were placed into 6-well plates containing pancreatic cancer cells and incubation was continued up to 3 days in RPMI1640 medium supplemented with 1% FBS, penicillin sodium and streptomycin sulfate.

2.5. miRNA microarray

Total RNAs including miRNAs were prepared using the miRNA RNeasy preparation kit (Qiagen, Valencia, CA). The Agilent's miRNA microarray (Human miRNA microarray Release 14.0; Agilent Technologies, Santa Clara, CA) containing probes for 904 human miRNAs was used to identify differentially expressed miRNAs between mono-cultured Panc-1 cells and those co-cultured with hPSC21-S/T cells. Data analysis was performed using the GeneSpring GX software version 12.5 (Agilent Technologies).

2.6. Quantitative real-time PCR for miRNA

Quantitative real-time PCR was performed using Taqman® Fast Universal PCR Master Mix (Life Technologies, Carlsbad, CA) and the StepOnePlus 7300 Real-Time PCR System (Life Technologies). Primers and probes were predesigned by the manufacturer (Life Technologies). The assay ID numbers were as follows: #000512 for hsa-miR-210 and #001093 for RNU6B. PCR was performed at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

The absolute number of copies was standardized by a sample standard curve. The values of hsa-miR-210 were normalized to RNU6B and expressed as those of the mono-cultured cells.

2.7. Inhibition of miR-210 expression

The stable miR-210 knockdown cell line was established by introducing tough decoy miRNA-blocking expression vector (pBAsi-Neo, Takara Bio, Otsu, Japan) which targets hsa-miR-210 using Lipofectamine 2000 (Life technologies) in Panc-1 cells. The control cell line was established by introducing tough decoy miRNA-blocking expression negative control vector (Takara Bio). Transfected cells were subjected to the limiting dilution in G418 containing (1 mg/ml) normal growth medium.

Transient inhibition of miR-210 expression was performed using the anti-miR-210 inhibitor (Cat#: AM10516; Life Technologies). Control experiments employed a negative control (Cat#: 17010; Life Technologies), Transfection was performed using the 4D-Nucleofector™ (Lonza, Basel, Switzerland) according to the manufacturer's instructions.

2.8. Western blotting

Cells were lysed, and total cell lysates (~100 μg) were fractionated on a 10% SDS–polyacrylamide gel. They were transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA), and the membrane was incubated overnight at 4 °C with rabbit anti-phospho-specific ERK antibody. After incubation with peroxidase-conjugated goat anti-rabbit *IgG* antibody, proteins were visualized using an enhanced chemiluminescence kit (GE Healthcare, Chalfont St Giles, United Kingdom). The levels of total ERK, Akt (total and phosphorylated at Ser^{473}), snai-1, and GAPDH were determined in a similar manner.

2.9. Immunofluorescent staining

Immunofluorescent staining of vimentin and β-catenin was performed as previously described [19]. Pancreatic cancer cells were seeded (1×10^5 cells/well) on cover slips placed on the bottom of 6-well plates. After 48 h of indirect co-culture with hPSC21-S/T cells, the pancreatic cancer cells were fixed in methanol at −20 °C. After blocking with 10% normal goat serum, the cells were incubated with mouse anti-vimentin or anti-β-catenin antibody at 1:100 dilution overnight at 4 °C. After washing, the cells were incubated with Alexa $Fluora^{546}$-labeled goat anti-mouse *IgG* antibody (Life Technologies) for 1 h. After washing, the cells wereanalyzed for fluorescence using an all-in-one type fluorescent microscope (Bio-Zero BZ-9000; Keyence, Osaka, Japan). Nuclei were counterstained with 4′, 6-diamidino-2-phenylinodole (DAPI). Expression of HIF-1α was examined in a similar manner.

2.10. Cell proliferation assay

Cell proliferation was assessed using a commercial kit (Roche Applied Science, Penzberg, Germany). This is a colorimetric immunoassay based on the measurement of 5-bromo-2′-deoxyuridine

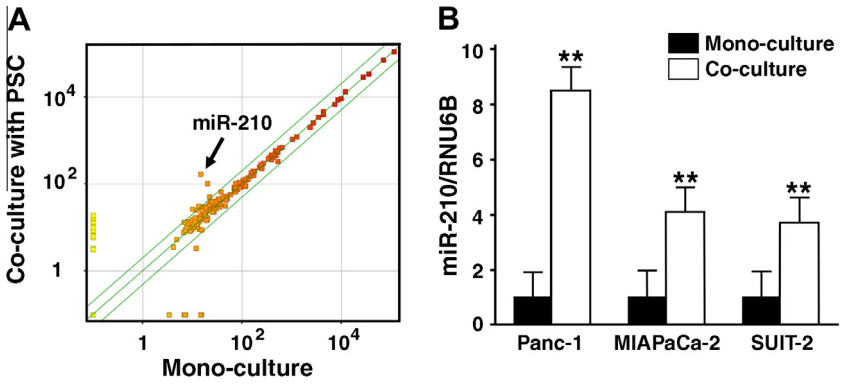

Fig. 1. Microarray analysis identified miR-210 as the most significantly upregulated miRNA in Panc-1 cells co-cultured with PSCs. Total RNAs including miRNAs were prepared from mono-cultured pancreatic cancer cells and those co-cultured with hPSC21-S/T cells for 72 h. (A) The miRNA expression profiles in the mono-cultured Panc-1 cells and those co-cultured with hPSC21-S/T cells were compared using the Agilent's miRNA microarray containing probes for 904 human miRNAs. A scatter plot of the microarray is presented. *X* and *Y* axes are shown in Log_{10} scale. Arrow denotes miR-210. (B) The levels of miR-210 and an internal control, RNU6B, were determined by real-time PCR. The values of miR-210 were normalized to RNU6B and expressed as those of the mono-cultured cells. $^{**}p < 0.01$ *vs.* mono-culture ($n = 6$).

(BrdU) incorporation during DNA synthesis. After 24-h incubation with PSC-CM, cells were labeled with BrdU for 3 h at 37 °C. Cells were fixed and incubated with peroxidase-conjugated anti-BrdU antibody. Then the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine was added, and BrdU incorporation was quantified by optical density (OD) 370-OD 492.

2.11. Two chamber assay

Cell suspension (5×10^4 cells/well) was added to the culture inserts with 8-μm pores in a 24-well companion plate (BD Biosciences). The lower chamber included 10% PSC-CM. After 24-h incubation, the cell suspension in the upper chamber was aspirated

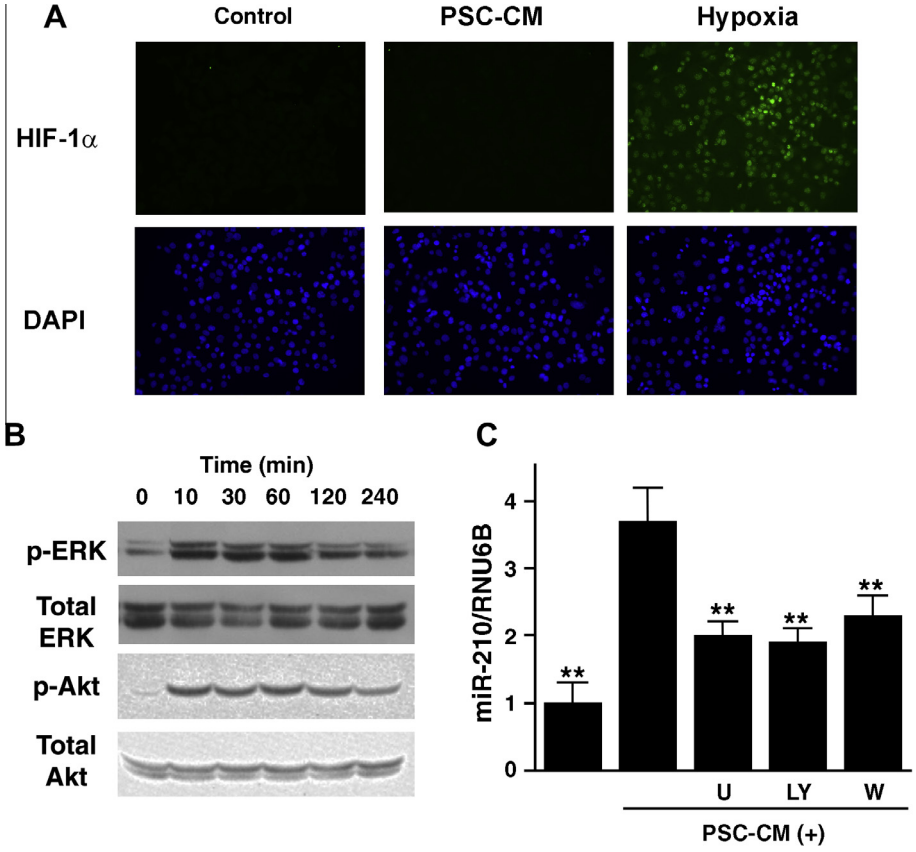

Fig. 2. PSCs increased miR-210 expression through the activation of ERK and PI3K/Akt pathways. (A) Panc-1 cells were left untreated under normoxia or hypoxia, or treated with 10% PSC-CM under normoxia for 4 h. The expression of HIF-1α was examined by immunofluorescent staining. Original magnification: X400. (B) Panc-1 cells were treated with 10% PSC-CM for the indicated time. Total cell lysates were prepared and the levels of ERK (phosphorylated at Tyr^{202}/Tyr^{204} and total) and Akt (phosphorylated at Ser^{473} and total) were examined by Western blotting. (C) Panc-1 cells were left untreated or treated with 10% PSC-CM in the absence or presence of inhibitors of signaling pathways. We employed U0126 (U, at 5 μM), LY294002 (at 10 μM), and wortmannin (W, at 100 nM). After 48-h incubation, total RNAs including miRNAs were prepared and the levels of miR-210 and RNU6B were determined by real-time PCR. The values of miR-210 were normalized to RNU6B and expressed as those of mono-cultured cells. $^{**}p < 0.01$ *vs.* 10% PSC-CM treatment ($n = 6$).

and the upper surface of the filter was carefully cleaned with cotton plugs. Cells that migrated through the polycarbonate membrane were stained with crystal violet and counted under a microscope.

2.12. Wound healing assay

Cells were grown to confluence, the cell monolayer was mechanically scarred with a sterile 200-μl pipette tip, and treated with PSC-CM up to 24 h.

2.13. Statistical analysis

The results were expressed as mean ± SE. Experiments were performed at least three times and similar results were obtained. Differences between the groups were evaluated by Student's *t*-test. A *P* value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Co-culture with PSCs upregulated miR-210 expression in pancreatic cancer cells

To identify miRNAs involved in the interaction between PSCs and pancreatic cancer cells, we compared the miRNA expression profiles between mono-cultured Panc-1 cells and those co-cultured with hPSC21-S/T cells using the Agilent's miRNA microarray. The microarray identified miR-210 as an upregulated miRNA in Panc-1 cells by co-culture with PSCs (11.7-fold, arrow in Fig. 1A). Other upregulated miRNAs included miR-146a-5p (5.2-fold), whereas miR-1238 was downregulated (0.28-fold). The increase in miR-210 expression was validated by real-time PCR. The expression of miR-210 was increased in all of the three pancreatic cancer cell lines examined (Panc-1, MIAPaCa-2, and SUIT-2 cells) by indirect co-culture with hPSC21-S/T cells compared with mono-culture (Fig. 1B). miR-210 is the most consistently and robustly upregulated miRNA under hypoxia in a wide variety of cell types [20,21]. Moreover, miR-210 acts as a "versatile" molecule affecting many aspects of the cellular responses to hypoxia [21]. miR-210 is currently considered the master 'microRNA' of the hypoxic response [22], and is known to influence physiological development as well as a number of hypoxia-dependent disease states. miR-210 mediates these functions by regulating many target mRNAs [23]. Previous studies have shown that the expression of miR-210 is increased and associated with poor prognosis in pancreatic cancer [13,16]. Aberrant miR-210 expression is not only present in solid tumors but also being secreted into the circulation, and can be detected in the plasma of patients with pancreatic cancer [15]. Because pancreatic cancer is characterized by an excessive desmoplastic reaction and by a hypoxic microenvironment within

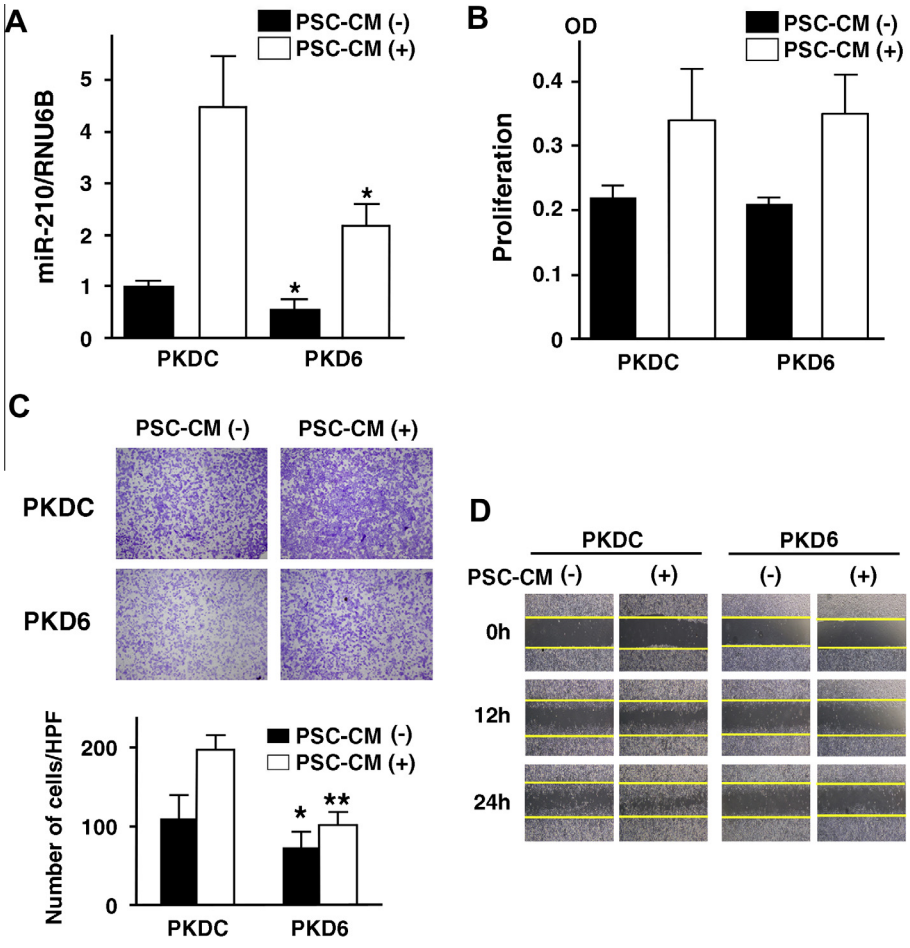

Fig. 3. Inhibition of miR-210 decreased migration, but not proliferation, of Panc-1 cells. (A, B) The miR-210-knockdown PKD6 and the control PKDC cells were left untreated or treated with 10% PSC-CM for 24 (A) or 48 (B) h. Expression of miR-210 was examined by real-time PCR. $^{*}p < 0.05$ ($n = 3$). (B) Cell proliferation was examined by the BrdU assay. Cell proliferation was not different between PKDC and PKD6 cells ($n = 6$). (C, D) Cell migration was examined by the two-chamber assay. After 24-h incubation with or without 10% PSC-CM, migrated cells were counted in random five high power fields (HPFs). $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* respective treatment in PKDC cells. (C) Representative photographs are shown. Original magnification: 40 times. (E) Cell monolayer was mechanically scarred with a pipette tip. Cells were left untreated or treated with 10% PSC-CM up to 24 h. Representative photographs at 0, 12 h, and 24 h are shown. Bars show the initial scratched area. Original magnification: 40 times.

the solid tumor mass [3,4,24], it is reasonable to presume that the hypoxic microenvironment upregulates miR-210 expression in pancreatic cancer cells. In addition, we here showed for the first time that PSCs directly up-regulated miR-210 expression in pancreatic cancer cells. Our results suggest that PSCs could increase miR-210 expression in pancreatic cancer by at least two mechanisms: indirectly through the hypoxic microenvironment generated by the excessive production of extracellular matrix and directly through unidentified factors produced by PSCs. Of note, we have previously shown that hypoxia stimulates the activation and cell functions in PSCs [25]. Thus, the hypoxic environment also accelerates PSCs to increase miR-210 expression in pancreatic cancer cells.

3.2. PSC-CM induced miR-210 expression through ERK- and PI3K/Akt-dependent pathways

Previous studies have shown that HIF-1α plays a central role in miR-210 expression in response to hypoxia [20]. To clarify the role of HIF-1α in PSC-induced miR-210 expression, we first examined whether PSC-CM induced HIF-1α expression in pancreatic cancer cells. Nuclear HIF-1α expression was induced by the hypoxic treatment but not by PSC-CM (Fig. 2A). In addition to HIF-α1, the roles of several signaling pathways in miR-210 induction have been reported [26–28]. For example, Akt activation facilitates the hypoxia-associated accumulation of miR-210 in an HIF-independent manner in cardiomyocytes [26]. ERK activation regulates reactive oxygen species-induced miR-210 upregulation in adipose-derived stem cells [27]. We therefore examined whether PSC-CM activated these signaling pathways. PSC-CM induced the activation of ERK and PI3K/Akt pathways, as shown by Western blotting using phospho-specific antibodies (Fig. 2B). miR-210 expression induced by PSC-CM was inhibited by the inhibitors of MEK/ERK (U0126) and PI3 K/Akt (LY294002 and wortmannin) pathways (Fig. 2C), suggesting roles of these signaling pathways in miR-210 induction.

3.3. Inhibition of miR-210 expression inhibited migration, but not proliferation, of Panc-1 cells

It has been reported that PSCs promote cancer cell progression by multiple mechanisms such as increased proliferation, migration and metastasis, and by protecting cancer cells from the induction of gemcitabine- and radiation-induced apoptosis [5–10]. To clarify the miR-210-regulated cell functions in pancreatic cancer cells, we established a stable miR-210 knockdown cell line. The expression of miR-210 was lower in the miR-210-knockdown cell line (PKD6) than that in the control cell line (PKDC) both in the absence and presence of PSC-CM (Fig. 3A). We first examined the effect of miR-210 inhibition on proliferation. PKD6 and PKDC cells were left untreated or treated with 10% PSC-CM, and cell proliferation was examined by the BrdU assay. PSC-CM increased proliferation, but the effects were not different between PKD6 and PKDC cells (Fig. 3B). The lack of inhibitory effects on the proliferation by miR-210 inhibition in pancreatic cancer cells is consistent with a previous study showing that overexpression of miR-210 did not affect the growth of SU86.86 pancreatic cancer cells [21].

We next focused on migration. PSC-CM increased the migration of PKDC cells (Fig. 3C, D). But, the stimulatory effects of PSC-CM were less pronounced in PKD6 cells. Similar effects were shown by the scratch assay (Fig. 3E). Thus, our results suggest a role of miR-210 in PSC-induced migration in pancreatic cancer cells.

3.4. miR-210 regulated PSC-induced epithelial-mesenchymal transition (EMT)

We have previously shown that PSCs induced EMT in pancreatic cancer cells [28]. EMT is a developmental process that allows a

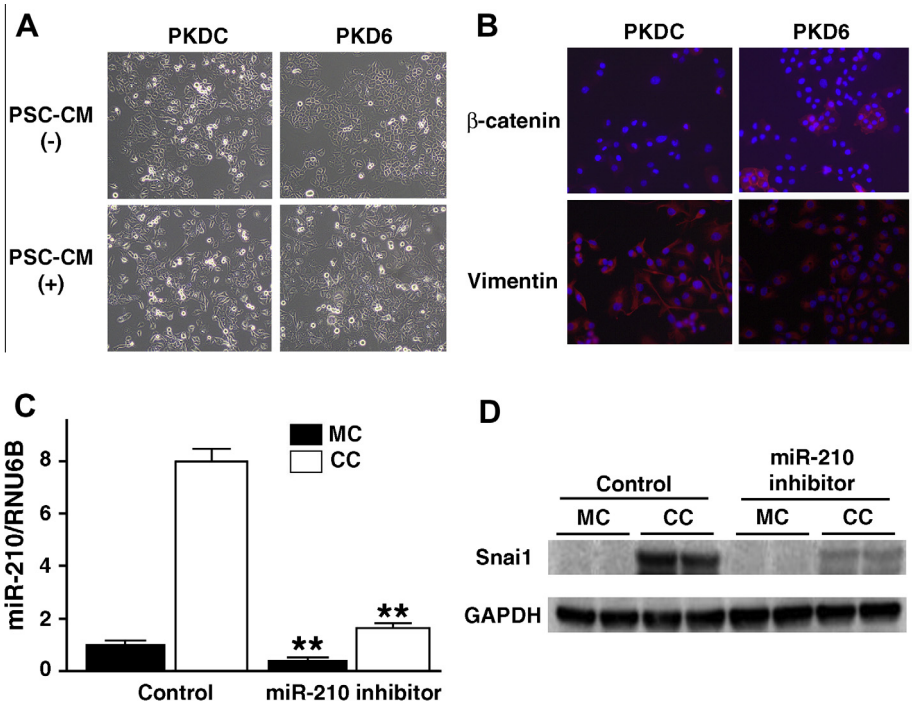

Fig. 4. Inhibition of miR-210 inhibited EMT-associated changes in Panc-1 cells. (A, B) PKDC and PKD6 cells were left untreated or treated with 10% PSC-CM for 48 h. (A) Untreated PKDC and PKD6 cells grew in monolayer cultures as epithelial clusters. PSC-CM induced loss of cell–cell contacts, spindle fibroblastic morphology, and cell scattering in PKDC cells. These PSC-CM-induced morphological changes were less pronounced in PKD6 cells. Original magnification: 40 times. (B) Expression of β-catenin and vimentin was examined in PSC-CM-treated PKDC and PKD6 cells. Nuclei were counterstained with DAPI. Original magnification: 400times. (C) Panc-1 cells were transfected with anti-miR-210 inhibitor or a negative control, and seeded in 6-well culture plates. From the next day, transfected Panc-1 cells were mono-cultured or co-cultured with hPSC21-S/T cells for 72 h. Total cell lysates were prepared and the expression of Snai1 and GAPDH was examined by Western blotting.

polarized epithelial cell to undergo multiple biochemical changes that enable it to assume a mesenchymal phenotype [29,30]. The EMT phenotype includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of extracellular matrix components [29,30]. EMT is now considered a critical process in cancer progression, and EMT induction in cancer cells results in the acquisition of invasive and metastatic properties as well as resistance to conventional therapies. Because increased cell migration is a feature of EMT, we examined whether the inhibition of miR-210 expression affected other phenotypes of EMT in pancreatic cancer cells. Although untreated PKDC and PKD6 cells grew in monolayer cultures as epithelial clusters, PSC-CM induced the loss of cell–cell contacts, spindle fibroblastic morphology, and cell scattering in PKDC cells (Fig. 4A). These PSC-CM-induced morphological changes were less pronounced in the PKD6 cells. We further examined the alterations of EMT markers in PKDC and PKD6 cells. PKDC cells treated with PSC-CM showed the cytoplasmic localization of β-catenin, whereas PKD6 cells treated with PSC-CM showed membrane-associated localization (Fig. 4B). This translocation of β-catenin from its usual membrane site to the cytoplasm is a characteristic phenomenon during the EMT [31]. Immunofluorescent staining showed that the PSC-CM-induced expression of the mesenchymal marker vimentin was lower in PKD6 cells than in PKDC cells (Fig. 4B). Western blotting showed that the expression of another mesenchymal marker, snai1 (the human homologue of *Drosophilia* Snail) was induced by the treatment with PSC-CM in PKDC cells, but the effect was less pronounced in PKD6 cells (Fig. 4C). Collectively, our results suggest a novel role of miR-210 in EMT in pancreatic cancer cells. To our knowledge, this is the first study showing the role of miR-210 in EMT. Along this line, Bao et al. [32] recently reported that a novel synthetic derivative of curcumin inhibited the expression of miR-210, miR-21, and EMT-associated molecules under hypoxia, supporting the association of miR-210 with EMT in pancreatic cancer cells.

Because PSCs play a critical role in the multiple steps of cancer progression, the interactions between PSCs and pancreatic cancer would serve as a novel therapeutic target for the treatment of pancreatic cancer. Accumulating evidence suggests that the extensive desmoplastic reaction is at least in part responsible for the innate resistance to chemotherapy and radiotherapy in pancreatic tumors by creating barriers that fence off tumor cells from circulating therapeutic compounds [33–35]. Breaching this stromal barrier could be a strategy to improve the delivery and efficacy of cytotoxic drugs in the future. Our results suggest that miR-210 might be a target for the treatment of pancreatic cancer. Experiments along this line are underway in our laboratory.

4. Conclusion

PSCs induced miR-210 expression in pancreatic cancer cells. Increased miR-210 expression might play a role in EMT. miR-210 might be a novel regulator of interactions between PSCs and pancreatic cancer.

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References

[1] R.E. Brand, M.A. Tempero, Pancreatic cancer, Curr. Opin. Oncol. 10 (1998) 362–366.
[2] A. Jemal, R. Siegel, E. Ward, et al., Cancer statistics, 2006, CA Cancer J. Clin. 56 (2006) 106–130.
[3] M. Hidalgo, Pancreatic cancer, N. Engl. J. Med. 362 (2010) 1605–1617.
[4] M.V. Apte, S. Park, P.A. Phillips, et al., Desmoplastic reaction in pancreatic cancer: role of pancreatic stellate cells, Pancreas 29 (2004) 179–187.
[5] R.F. Hwang, T. Moore, T. Arumugam, et al., Cancer-associated stromal fibroblasts promote pancreatic tumor progression, Cancer Res. 68 (2008) 918–926.
[6] A. Masamune, T. Shimosegawa, Signal transduction in pancreatic stellate cells, J. Gastroenterol. 44 (2009) 249–260.
[7] A. Masamune, T. Watanabe, K. Kikuta, et al., Roles of pancreatic stellate cells in pancreatic inflammation and fibrosis, Clin. Gastroenterol. Hepatol. 7 (2009) S48–54.
[8] M. Erkan, G. Adler, M.V. Apte, et al., StellaTUM: current consensus and discussion on pancreatic stellate cell research, Gut 61 (2012) 172–178.
[9] A. Masamune, T. Shimosegawa, Pancreatic stellate cells -multi-functional cells in the pancreas, Pancreatology 13 (2013) 102–105.
[10] M.V. Apte, J.S. Wilson, A. Lugea, et al., A starring role for stellate cells in the pancreatic cancer microenvironment, Gastroenterology 144 (2013) 1210–1219.
[11] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[12] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, Cell 136 (2009) 215–233.
[13] A.E. Szafranska, T.S. Davison, J. John, et al., MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma, Oncogene 26 (2007) 4442–4452.
[14] E.J. Lee, Y. Gusev, J. Jiang, et al., Expression profiling identifies microRNA signature in pancreatic cancer, Int. J. Cancer 120 (2007) 1046–1054.
[15] J. Wang, J. Chen, P. Chang, et al., MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease, Cancer Prev. Res (Phila) 2 (2009) 807–813.
[16] T. Greither, L.F. Grochola, A. Udelnow, et al., Elevated expression of microRNAs 155, 203, 210 and 222 in pancreatic tumors is associated with poorer survival, Int. J. Cancer 126 (2010) 73–80.
[17] I.G. Papaconstantinou, A. Manta, M. Gazouli, et al., Expression of microRNAs in patients with pancreatic cancer and its prognostic significance, Pancreas 42 (2013) 67–71.
[18] S. Hamada, A. Masamune, T. Takikawa, et al., Pancreatic stellate cells enhance stem cell-like phenotypes in pancreatic cancer cells, Biochem. Biophys. Res. Commun. 421 (2012) 349–354.
[19] K. Kikuta, A. Masamune, S. Hamada, et al., Pancreatic stellate cells reduce insulin expression and induce apoptosis in pancreatic β-cells, Biochem. Biophys. Res. Commun. 433 (2013) 292–297.
[20] Y.C. Chan, J. Banerjee, S.Y. Choi, et al., MiR-210: the master hypoxamir, Microcirculation 19 (2012) 215–223.
[21] X. Huang, L. Ding, K.L. Bennewith, et al., Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation, Mol. Cell 35 (2009) 856–867.
[22] M. Ivan, A.L. Harris, F. Martelli, et al., Hypoxia response and microRNAs: no longer two separate worlds, J. Cell Mol. Med. 12 (2008) 1426–1431.
[23] P. Fasanaro, S. Greco, M. Lorenzi, et al., An integrated approach for experimental target identification of hypoxia-induced miR-210, J. Biol. Chem. 284 (2009) 35134–35143.
[24] A.C. Koong, V.K. Mehta, Q.T. Le, et al., Pancreatic tumors show high levels of hypoxia, Int. J. Radiat. Oncol. Biol. Phys. 48 (2000) 919–922.
[25] A. Masamune, K. Kikuta, T. Watanabe, et al., Hypoxia stimulates pancreatic stellate cells to induce fibrosis and angiogenesis in pancreatic cancer, Am. J. Physiol. Gastrointest. Liver Physiol. 295 (2008) G709–G717.
[26] R.K. Mutharasan, V. Nagpal, Y. Ichikawa, et al., MicroRNA-210 is upregulated in hypoxic cardiomyocytes through Akt- and p53-dependent pathways and exerts cytoprotective effects, Am. J. Physiol. Heart Circ. Physiol. 301 (2011) H1519–H1530.
[27] J.H. Kim, S.G. Park, S.Y. Song, et al., Reactive oxygen species-responsive miR-210 regulates proliferation and migration of adipose-derived stem cells via PTPN2, Cell Death Dis. 11 (2013) e588.
[28] K. Kikuta, A. Masamune, T. Watanabe, et al., Pancreatic stellate cells promote epithelial-mesenchymal transition in pancreatic cancer cells, Biochem. Biophys. Res. Commun. 403 (2010) 380–384.
[29] J.P. Thiery, H. Acloque, R.Y. Huang, et al., Epithelial-mesenchymal transitions in development and disease, Cell 139 (2009) 871–890.
[30] R. Kalluri, R.A. Weinberg, The basics of epithelial-mesenchymal transition, J. Clin. Invest. 119 (2009) 1420–1428.
[31] D. Blanco, S. Vicent, E. Elizegi, et al., Altered expression of adhesion molecules and epithelial-mesenchymal transition in silica-induced rat lung carcinogenesis, Lab. Invest. 84 (2004) 999–1012.

[32] B. Bao, S. Ali, A. Ahmad, et al., Hypoxia-induced aggressiveness of pancreatic cancer cells is due to increased expression of VEGF, IL-6 and miR-21, which can be attenuated by CDF treatment, PLoS ONE 7 (2012) e50165.
[33] K.P. Olive, M.A. Jacobetz, C.J. Davidson, et al., Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer, Science 324 (2009) 1457–1461.
[34] T.S. Mantoni, S. Lunardi, O. Al-Assar, et al., Pancreatic stellate cells radioprotect pancreatic cancer cells through β1-integrin signaling, Cancer Res. 71 (2011) 3453–3458.
[35] C. Feig, A. Gopinathan, A. Neesse, et al., The pancreas cancer microenvironment, Clin. Cancer Res. 18 (2012) 4266–4276.