COL11A1 is the Key Target Gene of MIST1 on Tumor Progression of Pancreatic Cancer

Yang Li

Huazhong University of Science and Technology

Zhiqiang Liu

Huazhong University of Science and Technology

Ying Sun

Huazhong University of Science and Technology

Dianyun Ren

Huazhong University of Science and Technology

Yongfeng Li

Huazhong University of Science and Technology

Shanmiao Gou (✉ shanmiaogou@hust.edu.cn)

Huazhong University of Science and Technology

Research Article

Keywords: collagen type XI alpha 1 chain, class A basic helix-loop-helix protein 15, epithelial-mesenchyme transition, pancreatic cancer, therapeutic target.

Posted Date: August 31st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-793040/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: MIST1, a component of BHLH transcription factors, has been documented to be an important factor in tumor progression of pancreatic cancer, but the molecular mechanism is still unknown.

Methods: COL11A1 was screened as a candidate key target gene of MIST1 in pancreatic cancer by ChIP-seq assay and verified by RT-PCR and Western Blotting on MIST1-overexpression pancreatic cancer cells. ChIP and dual-luciferase assays were performed to study the binding domain of MIST1 and COL11A1. Transwell invasion, wound healing, MTT, colony formation assays and animal experiments were performed to investigate the roles of COL11A1 expression on pancreatic cancer cells. Clinical data and TCGA datasets were used to evaluate the role of COL11A1 expression on prognosis for patients with pancreatic cancer.

Results: MIST1 could bind to the promoter of COL11A1 as a negative transcription factor in pancreatic cancer. Overexpression of COL11A1 promotes pancreatic cancer cell growth, migration and invasion *in vitro* and *in vivo*. Expression of COL11A1 was upregulated in pancreatic cancer and positively correlated with a worse prognosis for patients with pancreatic cancer.

Conclusions: These results demonstrated COL11A1 as a carcinogen in pancreatic cancer, and it acts as the key target gene of MIST1 on tumor progression of pancreatic cancer. COL11A1 can act as a potential therapeutic target of pancreatic cancer which is superior to MIST1.

Background

MIST1, known as a basic helix-loop-helix (BHLH) transcription factor, regulates downstream genes by binding to the E-box sequence. MIST1 is essential for exocrine pancreas maturation and zymogen secretion [1, 2]. MIST1-silenced acinar cells fail to establish an apical-basal organization and generate zymogen granules or intercellular communications [3]. Recently, MIST1 was identified to be responsible for acinar-to-ductal metaplasia in pancreas [4]. In the absence of MIST1, the acinar cells were more likely to convert to ductal phenotype which led to the susceptibility of pancreatic cancer [5]. Our previous studies suggested overexpression of MIST1 reversed the EMT and reduced the tumor progression of pancreatic cancer cells [6].

Collagen type XI alpha 1 chain (COL11A1) belongs to the collagen family and forms one of the two alpha chain of collagen type XI, acting as a major component of the interstitial extracellular matrix (ECM) [7]. COL11A1 was first identified in cartilage and several non-cartilaginous tissues, such as bone, vitreous humor, skin, heart, sternum, and arterial smooth muscle cells [8]. Recently, several studies documented that the upregulation of COL11A1 plays important roles on malignancy of various tumors [9–12]. Here, we identified COL11A1 as the key target gene of MIST1 on EMT and tumor progression of pancreatic cancer. COL11A1 positively correlates with the progression and poor prognosis of pancreatic cancer, and expression of COL11A1 positively correlates with EMT and tumor progression of pancreatic cancer cells.

Downregulation of COL11A1 rescues the roles of downregulation of MIST1 on EMT and tumor progression of pancreatic cancer cells. These results suggests COL11A1 to be a potential therapeutic target for pancreatic cancer.

Materials And Methods

Human pancreatic tissue samples

pancreatic cancer and paracancerous tissue samples (n = 12) were obtained from patients at the Union Hospital of Huazhong University of Science and Technology (HUST). All tissues were stored immediately in liquid nitrogen after resection. The study was approved by the Ethics Committee at Tongji Medical College, HUST, China and written informed consent was obtained from patients before surgery as described before [6].

Cell culture

Human pancreatic cancer cell lines PANC-1, BxPC-3, and SW1990 were obtained from the ATCC (Manassas, VA, USA). PANC-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, UT, USA), and BxPC-3 and SW1990 cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% FBS. All Cells were cultured in 5% CO₂ at 37 °C.

Western Blotting and antibodies

Cells were washed with phosphate-buffered saline (PBS) three times and lysed in radioimmunoprecipitation assay (RIPA) buffer containing 1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) and 1% Halt phosphatase inhibitor cocktail (Thermo Scientific) for 30 min. After quantification using a protein quantification kit (Sigma-Aldrich), the same amounts of protein were separated using 10% SDS-PAGE gels. The proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Pierce Biotechnology, Rockford, IL, USA) and blocked using 5% skim milk for 1 h, before being incubated overnight at 4 °C with specific primary antibodies, followed by incubation with secondary antibodies (BOSTER, Pleasanton, CA, USA) for 1 h at room temperature. The membranes were imaged using the Molecular Imager Chemi Doc XRS⁺ imaging system (Bio-rad, Hercules, CA, USA) using ECL detection reagents (Thermo Fisher Scientific). The anti-MIST1 antibody (1:1000) was purchased from Cell Signaling Technology (Danvers, MA, USA); the anti-COL11A1 (1:1000) antibody was from Abcam (Cambridge, MA, USA). The anti-E-cad, N-cad, Vimentin, β -catenin, and Snail (1:1000) antibodies were from Cell Signaling Technology. The anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:3000) antibody was purchased from Proteintech (Rosemont, IL, USA).

Quantitative real-time reverse transcription PCR (qRT-PCR)

Total RNA was extracted using the TRIzol Reagent (Invitrogen, Waltham, MA, USA) and reverse transcribed to cDNA using a PrimeScript[®] RT reagent Kit (Takara, Shiga, Japan). Next, the quantitative real-time reverse transcription PCR (qRT-PCR) step was performed using a SYBR Green PCR Kit (Takara). The fold-enrichment was obtained from three independent replicate experiments and was normalized to the expression of GAPDH.

Chromatin immunoprecipitation (ChIP) assay and the luciferase reporter assay

The ChIP assay was performed using a SimpleChIP[®] Plus Sonication Chromatin IP Kit (#56383, Cell Signaling Technology). According to the manufacturer's protocol, cells transfected with MIST1 overexpression lentiviruses were cross-linked using 37% formaldehyde. DNA fragments were broken into 100–500 bp using ultrasound and separated by agarose gel electrophoresis. Then, the protein-DNA complexes were incubated vertically with 2 mg of anti-MIST1 antibodies or IgG overnight at 4 °C. Then, protein G agarose beads were used to precipitate the cross-linked chromatin. Finally, the chromatin was isolated and purified for qPCR. The percent of the input was calculated by: $5\% \times 2^{(CT_{5\%Input} - CT_{Sample})}$.

For the luciferase reporter assay, PANC-1 cells were added to the wells of 24-well plates, and co-transfected with luciferase reporter plasmids (Ribo biology, Guangzhou, China) and MIST1 overexpression lentiviruses for 48 h. Then, the luciferase activities were measured using the dual-luciferase reporter assay system (Promega, Madison, WI, USA). Renilla luciferase activity was used as a control.

Overexpression and Knockdown

Lentiviruses for MIST1 overexpression were purchased from Genechem (Shanghai, China) and transfected into cells. Green fluorescent protein (GFP)-positive infected cells were selected using puromycin. The short hairpin RNA targeting COL11A1 and the plasmid overexpressing COL11A1 were procured from Sigma-Aldrich and Ribo-biology (Guangzhou, China), respectively. Cells were transfected with the shRNA or plasmids using Lipofectamine 3000 (Invitrogen) and Opti-MEM media (Invitrogen). 24 h after transfection, the medium was replaced with fresh DMEM containing 10% FBS.

Cell invasion and migration assay

For the Transwell assay, 1×10^4 cells were plated in 200 μ L of PBS-free medium into upper chamber of a 24-well Corning Costar insert with 8-mm pores and 600 μ L of DMEM with 30% FBS was added into the lower chamber. After incubation at 37 °C for 24 h, the cells that had moved into the lower chamber were

fixed using 4% paraformaldehyde (PFA) and stained with crystal violet. The chambers were photographed under a microscope and cells were counted in five random fields.

The wound healing assay was conducted in 6-well plates in which cells had grown to full confluence. Wounds (scratches) were made in the cell layers, which were photographed at 0, 24, 48, and 72 h after wounding to measure the wound.

Cell proliferation

For MTT assay, 3000 cells in 200 mL of medium were plated into wells of a 96-well plate and grow for 5 days. On each incubated day, 50 mL of MTT solutions were added to seven independent replicate wells and incubated in the dark for 2 hours. Then 500 mL DMSO were added to dissolve the formazan crystals. The absorbances were measured at 570 nm using a microplate reader.

For the colony formation assay, 500 cells were seeded in 6-well plates. At 7 days after plating, the cells were washed with PBS three times, fixed in paraformaldehyde for 30 minutes, and then stained using crystal violet. The plates were photographed, and the number of colony cells was counted.

Generation of pancreatic cancer xenografts model and pulmonary metastasis model in nude mice

Fifteen four-week-old male athymic nude mice (4-weeks old, Vitalriver, Beijing, China) were randomly divided into three groups ($n=5$ per group) for xenografts models. The xenografts models were established by subcutaneously inoculating 5×10^6 PANC-1 cells infected with sh-control, COL11A1 or sh-COL11A1 lentiviruses diluted in 100 mL of PBS. Tumor volumes were calculated using the formula: tumor volume (mm^3) = (Length $\times$ Width²)/2. All the mice were sacrificed at day 21 after injection. Ten four-week-old male athymic nude mice were randomly divided into two groups ($n=5$ per group) for pulmonary metastasis models. The pulmonary metastasis models were generated by intravenously injecting 5×10^6 cells infected with sh-control or COL11A1 lentiviruses diluted in 100 mL of PBS into the tail. Hematoxylin and eosin (H&E) staining was performed to detect the lung metastatic foci. Yang Li was aware of the group allocation at the different stages of the experiment. The animal experiments were conducted under the guidelines approved by the local ethics committee (Tongji Medical College, HUST, China) and carried out in compliance with the Animals in Research: Reporting In vivo Experiments (ARRIVE) guidelines.

Statistical analysis

All data are expressed as the mean $\pm$ SD and were analyzed using GraphPad Prism 6 software (GraphPad Inc., La Jolla, CA, USA). Paired t-tests and one-way analysis of variance (ANOVA) were used to assess

statistical significance, which was set at $p < 0.05$. The clustering analysis was performed to group similar items together.

Results

COL11A1 expression is regulated by MIST1 via mRNA translation

We performed a Chip-seq assay using PANC-1-MIST1 cells designed to detect the direct targets of MIST1. The Chip-seq data were analyzed within ± 10 kb of the transcription start site (TSS), and 233 candidate genes directly regulated by MIST1 were pooled out [6]. The candidate genes enriched in 16 signaling pathways (Fig. 1a), and COL11A1 was a candidate gene in focal adhesion signaling pathway. To probe the gene correlation of in pancreatic cancer, we analyzed the TCGA datasets. Gene-Correlation analysis showed that MIST1 expression was statistical negatively correlated with COL11A1 expression in pancreatic cancer samples (Fig. 1b). Next, we verified whether MIST1 regulates COL11A1 expression in pancreatic cancer cells. MIST1 was stably overexpressed in PANC-1, BxPC-3 and SW1990 cell lines via lentivirus-mediated transduction. The COL11A1 expression decreased in pancreatic cancer cells overexpressing MIST1 (Fig. 1c, 1d).

The MIST1 binding site motif was shown in Fig. 1e according to the JASPAR database, and a ChIP assay verified this predicted binding (Fig. 1f). A luciferase reporter assay was then performed to assess the role of MIST1 on the COL11A1 promoter region activity. As shown in Fig. 1g, upregulating MIST1 expression significantly decreased the luciferase activity induced by the COL11A1 promoter, while binding site mutation abrogated this effect.

The N-terminal domain of MIST1 interacts with COL11A1 and regulates its translation

MIST1 has been reported to have a repression domain in its N-terminus. According to the Uniprot database, we identified amino acids 72–143 of MIST1 as a classical BHLH domain (Fig. 2a). Based on this, we generated a mutant MIST1 with an N-terminal amino acid deletion. To test the DNA binding function of the MIST1 classical BHLH domain, we also performed a ChIP assay for the mutant MIST1 (Fig. 2b). As shown in Fig. 2c, COL11A1 expression was recovered in MIST1-Mut-overexpressing cells compared with that in MIST1-WT-overexpressing cells. A luciferase reporter assay was then performed to confirm the role of the N-terminus of MIST1, results showed that N-terminus deleted MIST1 could not repress the transcription activity (Fig. 2d).

COL11A1 expression elevated in pancreatic cancer and high levels of COL11A1 correlated with poor prognosis

We used the GEPIA web tool to analyze the expression of COL11A1 in pancreatic cancer and non-tumor tissues matching The Cancer Genome Atlas (TCGA) normal and Genotype-Tissue Expression (GTEx) data. The expression of COL11A1 elevated significantly in pancreatic cancer compared with that in matched normal tissues (Fig. 3a). Next, we analyzed the mRNA and protein level of COL11A1 in 12 pairs

of tumor tissues and paracancerous tissues by qRT-PCR and western blotting. The significant higher expression of COL11A1 in tumor tissues than that in paracancerous tissues was observed in eight pairs of samples (Fig. 3b, c). The survival analysis based on TCGA datasets showed that high COL11A1 expression correlated with poor prognosis of pancreatic cancer (Fig. 3d).

COL11A1 knockdown inhibits pancreatic cancer cell proliferation, migration and invasion

COL11A1 was knockdown in PANC-1, BxPC-3 and SW1990 cell lines via shRNA. COL11A1 expression was significantly downregulated in transfected PANC-1 BxPC-3 and SW1990 cells compared to cells transfected with the control shRNA (Fig. 4a, 4b). Transwell invasion assays indicated knockdown of COL11A1 dramatically inhibited the invasion ability of pancreatic cancer cells (Fig. 4c). Wound healing assays showed knockdown of COL11A1 inhibited the migration ability of pancreatic cancer cells (Fig. 4d). MTT assays and colony formation assays revealed knockdown of COL11A1 reduced the proliferation of pancreatic cancer cells (Fig. 4e, 4f).

Overexpression of COL11A1 promotes tumor progression in vitro

The level of COL11A1 was elevated in PANC-1, BxPC-3, and SW1990 cell lines after transfection of the COL11A1-overexpressed plasmid. The transfection efficiency was verified by western blotting and qRT-PCR (Fig. 5a, b). The transwell and wound healing assays showed that the migration and invasion abilities were strengthened in COL11A1-overexpressed cells (Fig. 5c, d). Meanwhile, the MTT and colony formation assays indicated that COL11A1 overexpression enhanced cell proliferation and colony formation (Fig. 5e, f).

COL11A1 expression correlates with pancreatic xenograft tumor growth and dissemination to the liver in vivo

To assess the impact of COL11A1 in pancreatic cancer in vivo, we subcutaneously injected PANC-1-COL11A1, PANC-1-shCOL11A1 or PANC-1-shControl cells into the right flank or caudal vein of 4-week-old BALB/c nude mice. In the tumor xenograft model, the tumor growth rate and tumor weight were significantly higher in the PANC-1-COL11A1 group than the PANC-1-shControl group, while that were significantly lower in the PANC-1-shCOL11A1 group than the PANC-1-shControl group (Fig. 6a, b). In the model of dissemination to the liver, and the number of liver metastases was significantly higher in the PANC-1-COL11A1 group than the PANC-1-shControl group (Fig. 6c, d).

Overexpression of COL11A1 rescues the inhibition of pancreatic cancer cell proliferation, migration and invasion by overexpression of MIST1

Given that MIST1 suppresses COL11A1 expression in pancreatic cancer cells, we next validated whether COL11A1 is the key target gene of MIST1 on cell proliferation, migration and invasion. As shown in Fig. 7a-d, overexpression of COL11A1 could apparently reverse the inhibition of pancreatic cancer cell migration and proliferation by overexpression of MIST1. Our previous study showed that MIST1 regulates mesenchymal markers expression in pancreatic cancer cells. The present study revealed that

overexpression of COL11A1 also rescued the impact of overexpression of MIST1 on mesenchymal markers expression in pancreatic cancer cells using Western blotting (Fig. 7e).

Discussion

Our previous studies suggested overexpression of MIST1 reversed the EMT and reduced the tumor progression of pancreatic cancer cells. Here, we report that COL11A1 is the key target gene of MIST1 on tumor progression of pancreatic cancer. The expression of COL11A1 correlated with tumor progression of pancreatic cancer cells, and high expression of COL11A1 in pancreatic cancer led to a relatively poor survival in patients. COL11A1 may represent a novel potential therapeutic target for pancreatic cancer which may be superior to MIST1.

MIST1 is a bHLH transcription factor that regulates its target genes by binding to an E-box sequence. MIST1 has been documented to be a crucial factor required for the development, tissue repair and inflammation of the pancreas and the stomach [13–15]. It maintains the structural features of exocrine pancreatic cells, including cytoskeletal organization, cell polarity, and cell-to-cell communication [1, 3, 5, 16, 17]. Silencing MIST1 led to significant de-differentiation, greatly accelerating the progress of pancreatic intraepithelial neoplasia, eventually giving rise to pancreatic cancer[5]. We previously showed that overexpression of MIST1 reverses the EMT and reduces the tumor progression of pancreatic cancer cells. We next screened its key target gene for these roles, and the results of Chip-seq assay showed that COL11A1 was one of the 233 candidate genes directly regulated by MIST1 [6].

COL11A1 is a minor fibrillar collagen whose importance on the progression, metastasis, and drug resistance in cancer has been highlighted in recent research[18, 19]. Thus we used bioinformatics methods and molecular biology methods to confirm whether COL11A1 was regulated by MIST1 in pancreatic cancer. Results showed that COL11A1 was upregulated in pancreatic cancer, and COL11A1 expression negatively correlated to MIST1 expression. Results of luciferase reporter assay confirmed that MIST1 inhibited COL11A1 promoter region activity. Lacking a classical active transcription domain, MIST1 instead contains a repressive N-terminal region capable of inhibiting transcription[20–22]. According to the UniProt web tool, 1–75 amino acid region of MIST1 acts as its Nterminal inhibitory domain. We constructed a Mutant-MIST1 lacking amino acids 1–75. Functional experiments and luciferase reporter assays confirmed that MIST1 lacking N-terminus failed to inhibit the expression of COL11A1, which suggested that the N-terminal domain of MIST1 interacts with COL11A1 and regulates its translation.

Functional experiments confirmed the roles of COL11A1 expression on pancreatic cancer cell migration, invasion and proliferation in vitro and pancreatic xenograft tumor growth and dissemination to the liver in vivo, reversely to the effects of MIST1 expression in pancreatic cancer cells. We next verify whether COL11A1 overexpression rescued the effects of MIST1 overexpression in pancreatic cancer cells in vitro. Results showed that overexpression of COL11A1 rescued the effects of overexpression of MIST1 in pancreatic cancer cells, and overexpression of MIST1 in COL11A1-overexpressing pancreatic cancer cells

just slightly reduced cell migration, invasion, and proliferation in vitro. These results suggested COL11A1 is the key target gene of MIST1 on tumor progression of pancreatic cancer.

Our previous study demonstrated that MIST1 regulated EMT of pancreatic cancer cells via the Snail/E-cadherin pathway, thus we also investigated whether COL11A1 expression affects epithelial and mesenchymal marker expression. Interestingly, COL11A1 overexpression partly rescued the effects of MIST1 overexpression on epithelial and mesenchymal marker expression. The molecular mechanism was unknown. A pan-cancer gene expression study shows that several genes of COL11A1-correlated gene set are drivers of the metastatic program and EMT [23]. Zhang et al. demonstrated that COL11A1 modulates EMT of esophageal squamous cells via the intracellular AKT/ERK/c-Myc cascades [24]. Tu et al. [50] demonstrate that COL11A1 knockdown in non-small cell lung cancer cells decreases the expression of EMT markers vimentin and Snail as well as activities of Akt and ERK [25]. Another group show that COL11A1 enhanced cell proliferation, migration, invasion, and cisplatin resistance of non-small cell lung cancer cells via the Smad pathway [26]. The molecular mechanism of COL11A1 expression on epithelial and mesenchymal marker expression in pancreatic cancer need further studies.

To our knowledge, this is the first study to indicate that COL11A1 is the key target gene of MIST1 on tumor progression of pancreatic cancer. Considering that targeting MIST1, which is a transcription factor, may be with high risk of off-target effects for treatment of pancreatic cancer, COL11A1 should be a better target. However, it is difficult to clarify the effect of COL11A1 expression on epithelial and mesenchymal marker expression. Further studies are required to identify and characterize the mechanism.

Conclusions

In conclusion, we demonstrate COL11A1 functions as a tumor promotor in pancreatic cancer. COL11A1 mediates the roles of MIST1-downregulation on tumor progression of pancreatic cancer, and COL11A1 knockdown inhibits pancreatic cancer cell proliferation, migration and invasion. COL11A1 may represent a novel potential therapeutic target for pancreatic cancer.

Abbreviations

COL11A1	collagen type XI alpha 1 chain
MIST1	muscle, intestine and stomach expression 1
OS	Overall survival
GEPIA	Gene Expression Profiling Interactive Analysis
ChIP	Chromatin immunoprecipitation
TCGA	The Cancer Genome Atlas

GTEX	Genotype-Tissue Expression
EMT	Epithelial-mesenchyme transition
PanIN	Pancreatic intraepithelial neoplasia
ECM	Extracellular matrix

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee at Tongji Medical College, HUST, China.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests" in this section.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 81472309).

Author Contributions

YL and ZL performed the experiments; YS, YL and DR collected the data; SG wrote the paper and analyzed the data. All authors read and approved the final manuscript.

References

1. Pin CL, Rukstalis JM, Johnson C, Konieczny SF: **The bHLH transcription factor Mist1 is required to maintain exocrine pancreas cell organization and acinar cell identity**. *The Journal of cell biology* 2001, **155**(4):519-530.
2. Fazio EN, Everest M, Colman R, Wang R, Pin CL: **Altered Glut-2 accumulation and beta-cell function in mice lacking the exocrine-specific transcription factor, Mist1**. *The Journal of endocrinology* 2005,

187(3):407-418.

3. Direnzo D, Hess DA, Damsz B, Hallett JE, Marshall B, Goswami C, Liu Y, Deering T, Macdonald RJ, Konieczny SF: **Induced Mist1 expression promotes remodeling of mouse pancreatic acinar cells.** *Gastroenterology* 2012, **143**(2):469-480.
4. Shi G, DiRenzo D, Qu C, Barney D, Miley D, Konieczny SF: **Maintenance of acinar cell organization is critical to preventing Kras-induced acinar-ductal metaplasia.** *Oncogene* 2013, **32**(15):1950-1958.
5. Shi G, Zhu L, Sun Y, Bettencourt R, Damsz B, Hruban RH, Konieczny SF: **Loss of the acinar-restricted transcription factor Mist1 accelerates Kras-induced pancreatic intraepithelial neoplasia.** *Gastroenterology* 2009, **136**(4):1368-1378.
6. Li X, Chen H, Liu Z, Ye Z, Gou S, Wang C: **Overexpression of MIST1 reverses the epithelial-mesenchymal transition and reduces the tumorigenicity of pancreatic cancer cells via the Snail/E-cadherin pathway.** *Cancer letters* 2018, **431**:96-104.
7. Raglow Z, Thomas SM: **Tumor matrix protein collagen Xla1 in cancer.** *Cancer letters* 2015, **357**(2):448-453.
8. Yoshioka H, Greenwel P, Inoguchi K, Truter S, Inagaki Y, Ninomiya Y, Ramirez F: **Structural and functional analysis of the promoter of the human alpha 1(XI) collagen gene.** *The Journal of biological chemistry* 1995, **270**(1):418-424.
9. Wu YH, Chang TH, Huang YF, Chen CC, Chou CY: **COL11A1 confers chemoresistance on ovarian cancer cells through the activation of Akt/c/EBPβ pathway and PDK1 stabilization.** *Oncotarget* 2015, **6**(27):23748-23763.
10. Cheon DJ, Tong Y, Sim MS, Dering J, Berel D, Cui X, Lester J, Beach JA, Tighiouart M, Walts AE *et al*: **A collagen-remodeling gene signature regulated by TGF-β signaling is associated with metastasis and poor survival in serous ovarian cancer.** *Clinical cancer research : an official journal of the American Association for Cancer Research* 2014, **20**(3):711-723.
11. Wu YH, Chang TH, Huang YF, Huang HD, Chou CY: **COL11A1 promotes tumor progression and predicts poor clinical outcome in ovarian cancer.** *Oncogene* 2014, **33**(26):3432-3440.
12. Sok JC, Lee JA, Dasari S, Joyce S, Contrucci SC, Egloff AM, Trevelline BK, Joshi R, Kumari N, Grandis JR *et al*: **Collagen type XI α1 facilitates head and neck squamous cell cancer growth and invasion.** *British journal of cancer* 2013, **109**(12):3049-3056.
13. Min J, Vega PN, Engevik AC, Williams JA, Yang Q, Patterson LM, Simmons AJ, Bliton RJ, Betts JW, Lau KS *et al*: **Heterogeneity and dynamics of active Kras-induced dysplastic lineages from mouse corpus stomach.** *Nature communications* 2019, **10**(1):5549.

14. Hata M, Kinoshita H, Hayakawa Y, Konishi M, Tsuboi M, Oya Y, Kurokawa K, Hayata Y, Nakagawa H, Tateishi K *et al*: **GPR30-Expressing Gastric Chief Cells Do Not Dedifferentiate But Are Eliminated via PDK-Dependent Cell Competition During Development of Metaplasia.** *Gastroenterology* 2020, **158**(6):1650-1666 e1615.
15. Nienhuser H, Kim W, Malagola E, Ruan T, Valenti G, Middelhoff M, Bass A, Der CJ, Hayakawa Y, Wang TC: **Mist1+ gastric isthmus stem cells are regulated by Wnt5a and expand in response to injury and inflammation in mice.** *Gut* 2021, **70**(4):654-665.
16. Huh WJ, Esen E, Geahlen JH, Bredemeyer AJ, Lee AH, Shi G, Konieczny SF, Glimcher LH, Mills JC: **XBP1 controls maturation of gastric zymogenic cells by induction of MIST1 and expansion of the rough endoplasmic reticulum.** *Gastroenterology* 2010, **139**(6):2038-2049.
17. Martinelli P, Carrillo-de Santa Pau E, Cox T, Sainz B, Jr., Dusetti N, Greenhalf W, Rinaldi L, Costello E, Ghaneh P, Malats N *et al*: **GATA6 regulates EMT and tumour dissemination, and is a marker of response to adjuvant chemotherapy in pancreatic cancer.** *Gut* 2017, **66**(9):1665-1676.
18. Wu YH, Huang YF, Chang TH, Chen CC, Wu PY, Huang SC, Chou CY: **COL11A1 activates cancer-associated fibroblasts by modulating TGF-beta3 through the NF-kappaB/IGFBP2 axis in ovarian cancer cells.** *Oncogene* 2021, **40**(26):4503-4519.
19. Nallanthighal S, Heiserman JP, Cheon DJ: **Collagen Type XI Alpha 1 (COL11A1): A Novel Biomarker and a Key Player in Cancer.** *Cancers* 2021, **13**(5).
20. Lemerrier C, To RQ, Carrasco RA, Konieczny SF: **The basic helix-loop-helix transcription factor Mist1 functions as a transcriptional repressor of myoD.** *The EMBO journal* 1998, **17**(5):1412-1422.
21. Tran T, Jia D, Sun Y, Konieczny SF: **The bHLH domain of Mistl is sufficient to activate gene transcription.** *Gene expression* 2007, **13**(4-5):241-253.
22. Kolonko M, Greb-Markiewicz B: **bHLH-PAS Proteins: Their Structure and Intrinsic Disorder.** *International journal of molecular sciences* 2019, **20**(15).
23. Jia D, Liu Z, Deng N, Tan TZ, Huang RY, Taylor-Harding B, Cheon DJ, Lawrenson K, Wiedemeyer WR, Walts AE *et al*: **A COL11A1-correlated pan-cancer gene signature of activated fibroblasts for the prioritization of therapeutic targets.** *Cancer letters* 2016, **382**(2):203-214.
24. Zhang B, Zhang C, Yang X, Chen Y, Zhang H, Liu J, Wu Q: **Cytoplasmic collagen XIalpha1 as a prognostic biomarker in esophageal squamous cell carcinoma.** *Cancer biology & therapy* 2018, **19**(5):364-372.
25. Tu H, Li J, Lin L, Wang L: **COL11A1 Was Involved in Cell Proliferation, Apoptosis and Migration in Non-Small Cell Lung Cancer Cells.** *Journal of investigative surgery : the official journal of the Academy of Surgical Research* 2021, **34**(6):664-669.

26. Shen L, Yang M, Lin Q, Zhang Z, Zhu B, Miao C: COL11A1 is overexpressed in recurrent non-small cell lung cancer and promotes cell proliferation, migration, invasion and drug resistance. *Oncology reports* 2016, **36**(2):877-885.

Figures

Figure 1

COL11A1 expression is regulated by MIST1 via mRNA translation. a. Clustering analysis of genes regulated by MIST1 based on the ChIP-seq results in PANC-1. b. Relative expression of MIST1 mRNA correlated negatively with COL11A1 in TCGA datasets. c and d. Expression of COL11A1 was reduced in MIST1-overexpressing cells. e. The transcription binding site motif of MIST1 provided by the JASPAR database. f. Enrichment of MIST1 across the COL11A1 promote region in PANC-1 cells using ChIP assay. g. Luciferase assay in PANC-1 cells co-transfected with COL11A1 luciferase reporter plasmids and MIST1 overexpression plasmid. Values are mean $\pm$ SD, *P < 0.05, ***P < 0.01.

Figure 2

The N-terminal domain of MIST1 interacts with COL11A1 and regulates its translation. a. The amino acid sequence of MIST1. The sequence in the red box is a predicted inhibitory domain (rank 1 to 72), and the sequence in the blue box is the classic BHLH domain according to the UniProt web tool. b. Local enrichment of MIST1-Mut across the COL11A1 promote region in MIST1-Mut transfected PANC-1 cells and empty vector transfected cells using ChIP assay. c. MIST1-Mut lost the inhibitory effect on the expression of COL11A1. d. Luciferase assay in PANC-1 cells co-transfected with COL11A1 luciferase reporter plasmids and MIST1 overexpression plasmid or MIST1-Mut overexpression plasmid. Values are mean $\pm$ SD, *P < 0.05, ***P < 0.01.

Figure 3

COL11A1 expression elevated in pancreatic cancer and high levels of COL11A1 correlated with poor prognosis. a. COL11A1 was upregulated in pancreatic cancer tissues (T) compared with normal pancreatic tissues (N) in TCGA datasets. b and c. COL11A1 showed a trend of upregulation in pancreatic cancer tissues compared with normal pancreatic tissues in 12 paired pancreatic cancer tissues (T) and

normal tissues (N). d. COL11A1 expression negatively correlated with prognosis of pancreatic cancer in TCGA datasets. Values are mean $\pm$ SD, * $P < 0.05$.

Figure 4

COL11A1 knockdown inhibits pancreatic cancer cell proliferation, migration and invasion. PANC-1, BxPC-3 and SW1990 cells were transfected with lentiviruses containing a shRNA-COL11A1 expression vector or empty control vector. Quantification of MIST1 protein (a) and mRNA (b) expression. Cell migration was

evaluated using Transwell migration assays (c) and wound healing assays (d), cell growth and viability were evaluated using the MTT assay (e), colony formation ability was evaluated using plate cloning experiment (f). Values are mean $\pm$ SD, * P < 0.05, *** P < 0.01.

Figure 5

Overexpression of COL11A1 promotes pancreatic cancer cell proliferation, migration and invasion. PANC-1, BxPC-3 and SW1990 cells were transfected with lentiviruses containing a COL11A1 expression vector

or empty control vector. Quantification of MIST1 protein (a) and mRNA (b) expression. Cell migration was evaluated using Transwell migration assays (c) and wound healing assays (d), cell growth and viability were evaluated using the MTT assay (e), colony formation ability was evaluated using plate cloning experiment (f). Values are mean $\pm$ SD, * $P < 0.05$, *** $P < 0.01$.

Figure 6

COL11A1 expression correlates with pancreatic xenograft tumor growth and dissemination to the liver in vivo. a, b. Nude mice were subcutaneously injected with stably transfected PANC-1 cells. Tumor volumes were calculated every 3 days (tumor volume = length × width²/2). Tumor weight was determined 3 weeks after injection. c, d. Stably transfected PANC-1 cells were injected into the tail vein of 4-week-old male nude mice. Quantification of liver metastasis at 4 weeks after injection. Values are mean ± SD, *P < 0.05, ***P < 0.01.

Figure 7

Overexpression of COL11A1 rescues the inhibition of pancreatic cancer cell proliferation, migration and invasion by overexpression of MIST1. PANC-1 cells were transfected with shControl, shCOL11A1, Vector, or MIST1. Cell migration was evaluated using Transwell migration assays (a) and wound healing assays (b), colony formation ability was evaluated using plate cloning experiment (c), cell growth and viability were evaluated using the MTT assay (d), and EMT-related protein expression was evaluated using Western blotting (e). Values are mean $\pm$ SD, *P < 0.05, ***P < 0.01.