



A Disintegrin and Metalloprotease 10 Expressions Modulate Potential Metastatic and Thrombus Formation in Pancreatic Carcinoma

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(Received 15 Nov 2021; accepted 21 Jan 2022)

Abstract

Background: Clinical investigations repurposing a disintegrin and metalloproteases 10 (ADAM10) as metastatic and thrombus marker have achieved encouraging results, but the mechanism behind this association remains unclear.

Methods: This study was carried out in NingXia and Wuhan, China from 2017 to 2021. The effects of ADAM10 expression on the metastatic and thrombus-associated genes: tissue factor (*TF*), P-selectin glycoprotein ligand-1 (*PSGL-1*), cathepsin G (*CTSG*) and mucin 1 (*MUC1*) were examined by immunofluorescence, qRT-PCR and Western blotting analysis. Metastatic and thrombotic behaviors were evaluated using NODSCID mouse model.

Results: The ADAM10 expression controlled the migration and invasion of pancreatic carcinoma cell-1 (PANC-1), and significantly regulated the metastatic and thrombus-associated genes ($P < 0.05$). ADAM10 and MUC1 were regulated and aberrantly expressed by a dependent mechanism. Moreover, ADAM10 expression induced the progression of adenocarcinoma cells and thrombus formation in vivo.

Conclusion: Regulation of ADAM10 expression in cancer cells might effectively pave the way for a more potent anti-metastatic and anti-thrombotic approach and could regulate the invasion and migration of cancer cells.

Keywords: ADAM10 protein; Metastasis; Thrombosis



Introduction

Cancer cells communicate bidirectionally with the surrounding microenvironment, sending and receiving structural and functional signals that direct diverse cellular activities, including cell proliferation, differentiation, growth, invasion and metastasis(1). Metastatic diseases are considered as a primary cause of death in cancer patients. Depending on the tumor locations, tumor cells display diverse sets of complicated pathways essentially required for metastasis. Metastatic cancer cells initiate a cascade of events comprising a long series of sequential steps, and numerous factors supporting the tumor cells survival, intravasation into bloodstream, escaping immune surveillance, adherence to endothelial cells and extravasations of the circulatory system followed by angiogenesis and progressive colonies at a distant site.

Pancreatic adenocarcinoma cells are complex at the metabolic, genomic and epigenetic levels (2), with multiple complicated pathways. Due to high metastatic ability, drug resistance and thrombus formation, pancreatic cancer is becoming as one of the leading cause of cancer-associated mortality (3). Although the accumulation of V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) proto-oncogene, cyclin dependent kinase inhibitor 2A (CDKN2A), SMAD family member 4 (SMAD4) and tumor protein p53 (TP53) gene mutations contributes to pancreatic cancer development, some other factors, including the expression of adhesion, cytokines and inflammatory molecules on cancer cells (4), are thought to be implicated in the aggressiveness of pancreatic cancer cells. A disintegrin and metalloproteases 10 (ADAM10), a member of disintegrin family of zinc-dependent metalloproteases, is localized in the membrane of epithelial cells of benign glands and played a vital role in tumor cell interactions, survival and evading immune surveillance. ADAM10 is responsible for the shedding of several molecular markers such as Notch, epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor-2 (VEGFR-2), epi-

thelial cadherin (E-cadherin), L1 cell adhesion molecule (L1-CAM), erb-b2 receptor tyrosine kinase 2 (ERBB2) and cluster of differentiation 44 (CD44) (5) that control tumor cell differentiation, proliferation, adhesion, migration and invasion. In addition to these molecules, ADAM10 is known to be contributed in shedding of different chemotactic and inflammatory molecules, such as interleukin 6 (IL-6) receptor, tumor necrosis factor- α (TNF- α), CXC-chemokine ligand 16(CXCL 16), fractalkine and CD40L(6). It is not surprising that ADAM10 proteolytic activity have been reported in preventing the process of malignant transformation and tumor resistance. Therefore, ADAM10 regulates a delicate balance of signals, they vitally direct different cell processes. Although directing these intricate pathways and their dynamic interactions will help to identify promising molecular targets for metastasis and immunogenicity; there have been very few study focused on regulation of metastatic and thrombus-associated genes *TF*, *PSGL-1*, *CTSG* and *MUC1* in pancreatic cancer cells. Moreover, there are still infrequent studies concerning the expression and functional role of ADAM10 in adenocarcinoma cell lines.

The current study proposed that the expression of metastatic and thrombus-associated genes in pancreatic cancer cells could be tuned by regulating the expression levels of ADAM10. Furthermore, we assumed that (i) overexpression of aberrant ADAM10 induce the progression of adenocarcinoma cells and thrombus formation, (ii) silencing of ADAM10 in tumor cells inhibit their metastatic and thrombotic behaviors. We constructed a eukaryotic vector expressing *ADAM10* gene to investigate the biological activities and metabolic effects of ADAM10 overexpression in pancreatic adenocarcinoma cell-1(PANC-1). Moreover, siRNA was used to silence ADAM10 expression and to examine its effects on cancer cell growth, migration and thrombus-associated genes.

Materials and Methods

Ethics approval

All the cells and animal experiments were conducted in conformity with a method approved by the Ethics Committee of General Hospital of Ningxia Medical University (Ethical Number: 2017-024).

Cell line and culture conditions

The human PANC-1 were purchased from the Bank of Chinese Academy of Medical Sciences, Beijing, China. The cells were cultured in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum (FBS) (Gibco BRL, Life Technologies, USA), penicillin (100 U/ml), and streptomycin (100 µg/ml) (Biosharp, Shanghai, China). Cells were incubated at 37°C in humidified air with 5% CO₂.

Total RNA extraction and quantification

Total RNA was isolated from the cell lines using RNAiso Plus based on the manufacturer's instructions. RNA concentrations were measured with a spectrophotometer (Life Technology, USA). The isolated samples were characterized by an A260/A280 ratio >1.90. cDNA was synthesized using the Transcriptor First Strand cDNA Synthesis Kit (Takara Bio, USA). The products were amplified using PCR in the presence of appropriate primers (Sangon Biotech, Shanghai, China), and the following PCR conditions were used for amplification: denaturation at 95 °C for 1 min; 40 cycles at 95 °C for 15 sec, 58 °C for 20 sec, and 72 °C for 45 sec; and final primer extension at 60 °C for 5 min.

ADAM10 cDNA synthesis

One µg of total RNA was utilized to form ADAM10 with High Fidelity Prime Script™ RT-PCR Kit (TaKaRa). Then a pair of primers: forward 5'-ATGGAGGGGTCTAAGGCGTCCAGC-3' and reverse 5'-GTGTCCTCACAGTTCTATAACAAG-3' were designed to contain restriction enzyme cutting sites.

Purification of ADAM10 cDNA and construction of recombinant pcDNA 3.1(+)/ADAM10

The products of RT-PCR were separated using agarose gel electrophoresis (1.5%). The double EcoRI and XhoI restriction enzymes were used to digest the DNA plasmids. The target fragments (ADAM10 cDNAs) were retrieved and isolated using Agarose Gel DNA Purification Kit v.2.0 (TaKaRa). The fragments were then polyadenylated using DNA A-Tailing Kit and ligated into pcDNA 3.1(+) vector using DNA Ligation Kit v.2.0 (TA Clone). Competent yeast cells were used to amplify the recombinant pcDNA 3.1 (+)/ADAM10. The pcDNA 3.1 (+)/ADAM10 was purified from transformants by using TaKaRa MiniBEST Plasmid Purification Kit v.2.0 and its correct sequence was confirmed by restriction enzyme mapping.

Establishment of PANC1 cells clones

The cells were divided into following groups: no treatment (NC), EV (control), recombinant pcDNA 3.1(+)/ADAM10, or transfected using 30 µl (containing 20 nM ADAM10 or MUC1 siRNA and 20 nM control siRNA) of transfection reagent (RNA Transfection Reagent, RiboBio) with four types of siRNA following the manufacturer's instructions. The effectiveness of mRNA knockdown was measured 48 h after transfection by qRT-PCR, and the protein was investigated by Western blotting.

The ADAM10 sequences are as follows: siRNA1 GCUGAUGAGAAGGACCCUAdTdT, siRNA2 GGUCUCAUGUACCUCCEAAAdTdT and siRNA3 GCACACCAGGAGAAUCU AAdTdT. A non-targeting scrambled siRNA GCUGUGAAAGGCACGACUAdTdT (Silencer, RiboBio) served as a negative control. The MUC1 siRNA sequences were as follows: siRNA1 GCACCCAGUCUCCUUUCUUDTdT, siRNA2 GCCUCUCCAAUAUUAAGUUDTdT, siRNA3 GCUAUCCCAGCACCGACUAdTdT and a non-targeting scrambled siRNA GCAACUGUCCCUUCCCCUUDTdT (Silencer, RiboBio) as a negative control.

Apoptosis detection assays

For apoptosis assessment, the cells were analyzed by flow cytometry after treated at room temperature with propidium iodide (PI)/annexin V-fluorescein isothiocyanate for 20 min.

Wound healing assay

After 48 h of cell transfection in a 6-well plate, the cells were at 90% confluency/well and scratched with a 200 μ l pipette tip. To remove detached cells, the plates were treated twice with PBS and incubated with complete growth medium without FBS. After the underside of the dish was marked, the initial photographs, as well as subsequent images over the next 24 h, were taken with an inverted phase microscope (40 \times magnification).

Transwell assay

Matrigel membranes (Matrigel Invasion Chamber, BD Bioscience) were incubated at 37 $^{\circ}$ C and 5% CO₂ for 2 h after addition of 500 μ l serum-free medium to rehydrate the plate. The lower compartment of the chamber was filled with the medium containing 10% FBS. After 48 h, the transfected cells were harvested and added (1 \times 10⁵ cells/well) to the upper chambers containing free medium. After incubation for 24 h, the chemotaxis assay was stopped and the non-invading cells were detached from the membrane by a cotton swab. Then, the membranes were placed in ice-cold methanol for 20 min, and the cells were treated with 0.1% crystal violet for 10 min at RT. The migrated cells were detected under a microscope (magnification, \times 200).

Fluorescence microscopy

Cells were treated with PBS, and fixed in 2% paraformaldehyde for 20 min at 4 $^{\circ}$ C. Then were incubated with 0.3% Triton X-100 (Santa Cruz Biotechnology, California, USA) for 10 min and blocked for 1 h at 4 $^{\circ}$ C with 5% BSA solution. Cells were incubated overnight at 4 $^{\circ}$ C with the appropriate dilution of primary antibodies. The samples were incubated with an Alexa Fluor 594-conjugated secondary antibody (red) (AntGene Biotechnology, Wuhan, China) for 1 h and nuclei

were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (50 μ M) for 10 min. Fluorescence image was detected by confocal microscopy (Nikon with inverted microscope). Controls omitting primary or secondary antibodies confirmed no labeling.

Western blotting

Whole-cell extracts were harvested with radio-immunoprecipitation assay (RIPA) solution (Fermentas, USA) to isolate total protein from control and transfected cell lines PANC1. The protein concentration was measured using the micro-BCA protein assay (ASPEN, AS1086). The protein lysates were separated by electrophoresis in appropriate concentration of Bis-Tris gels (ASPEN), transferred onto a polyvinylidene difluoride (PVDF, 0.45 μ m) (Millipore, IPVH00010) membrane, and blocked for 1 h with non-fat dry milk (5%) in TTBS (5% TBS, 20 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20) at room temperature. GAPDH was utilized as internal control. The PVDF membranes were probed with specific primary antibodies overnight at 4 $^{\circ}$ C, washed with TTBS and blocked for 2 h at RT with the appropriate secondary antibody. The membranes were imaged and the density of each band was analyzed using GelDoc software (Bio-Rad, Munich, Germany).

Tumor formation assay using NODSCID mice

Cultured PANC1 cells transfected with negative control siRNA, ADAM10 siRNA; or recombinant pcDNA 3.1(+)/ADAM10 or EV were digested and diluted to 50 μ l cell suspension (2 \times 10⁷ cells/ml). 24 NODSCID mice were divided into 4 groups averagely and randomly, followed by subcutaneous injection of the right lateral of the mice without anesthesia.

Mice were maintained in respective cages under pathogen-free conditions with standard food and water. Tumor size (mm³) was measured by calculating $V = W^2 \times L / 2$ in which W corresponds to the width (in mm) and L to the tumor length (in mm) or using a vernier caliper. Then the mice were sacrificed at 33 days after inoculation.

Analysis of lung tissues

Mice lungs were collected, stored in 10% formalin overnight, immersed in 30% sucrose for 48 h and then in Optimal Cutting Temperature (OCT) overnight. The immersed lungs in OCT were frozen and then sectioned (14-mm sections) by using Leica CM1905 cryostat. H&E staining was used to stain the lung sections (6 sections/ animal, n=6 mice per group). Finally, the slides were imaged with a microscope (ZeissAxio observer A1) at 2.5' magnification.

Statistical analysis

Data are presented as the mean ± SEM of at least triple experiments. The differences between groups were evaluated by ANOVA with Tukey's post-hoc test or Student's *t* test using GraphPad Prism8 Software. The two-sided probability level *P* < 0.05 was considered statistically significant. Differences with *P* < 0.05 are described as follows: **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

Results

Assessment of qRT-PCR product and pcDNA 3.1(+)/ADAM10 expression

After loading the products of RT-PCR on agarose gels (1.5%), a band of full-length ADAM10 cDNA was formed at 2500 bp. The fragment was inserted into the pcDNA 3.1(+) vector and confirmed by restriction enzyme mapping and electrophoresis. pcDNA 3.1(+)/ADAM10 was successfully formed, and ADAM10 was overexpressed in PANC1 cancer cells after transfection.

Effect of ADAM10 on pancreatic cancer cells behavior

To assess the effect of ADAM10 on pancreatic cancer cells behavior, we generated two types of PANC1 clones of expressing ADAM10 and silencing ADAM10 expression by using recombinant pcDNA 3.1(+)/ADAM10 vector and siRNA strategies (si1.1, si1.2 and si1.3 ADAM10), respectively.

To examine the functional consequences of ADAM10 expression on cell apoptosis, flow cytometric (Fig. 1) assay was performed. Compared to control group (Fig. 1A), the impact of overexpression of ADAM10 in adenocarcinoma cell lines showed slight increase in apoptotic rates (Fig. 1B), while the silencing showed a significant increase in apoptotic rates (Fig. 1C).

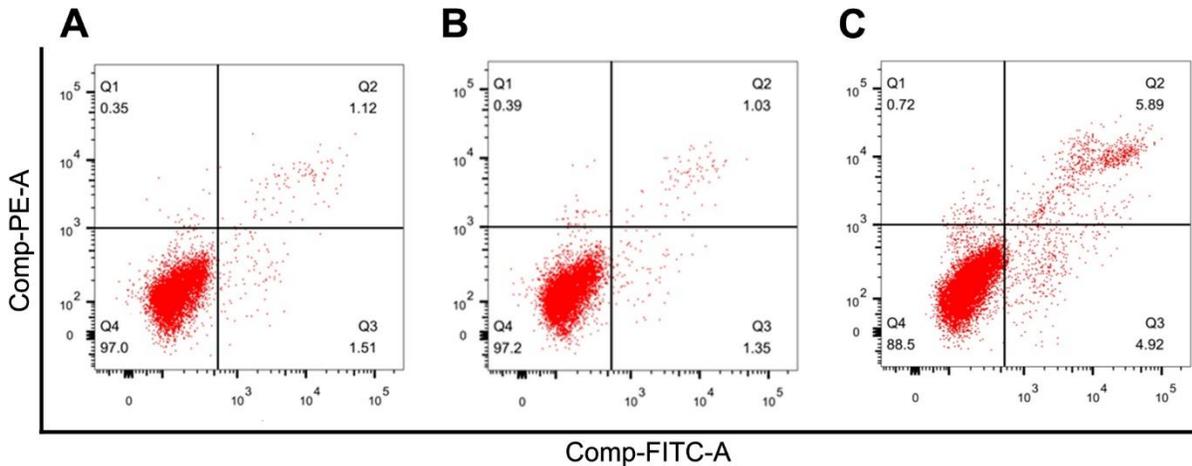


Fig. 1: Cell apoptosis in PANC1 cells determined with flow cytometry after transfection for 24 h. (A) Representative dot plots for non-treated PANC1 cells (control). (B) and (C) cell apoptosis rates of PANC1 cells were induced by pcDNA 3.1(+)/ADAM10 and ADAM10 siRNA, respectively

Then wound healing assay was performed to examine the effect on cell migration (Fig. 2A-D). We noticed a considerable enhancement in migration rates of PANC1 clones over-expressing ADAM10 (1.5-fold). In contrast, in ADAM10 silencing PANC1 clones, the total scratch size was reduced by 80% to 75% within 24 h compared to control cells.

To further investigate the role of ADAM10 in adenocarcinoma cell invasion, Transwell assay

was performed (Fig. 2E-H). The invasiveness properties of PANC1 cells was significantly enhanced by ADAM10 overexpression, compared to EV control cells ($P < 0.005$; Fig. 2E and G). In contrast, down-regulation of ADAM10 significantly reduced ($P < 0.001$) the invasiveness of PANC1 cells compared with scramble control (Fig. 2F and H).

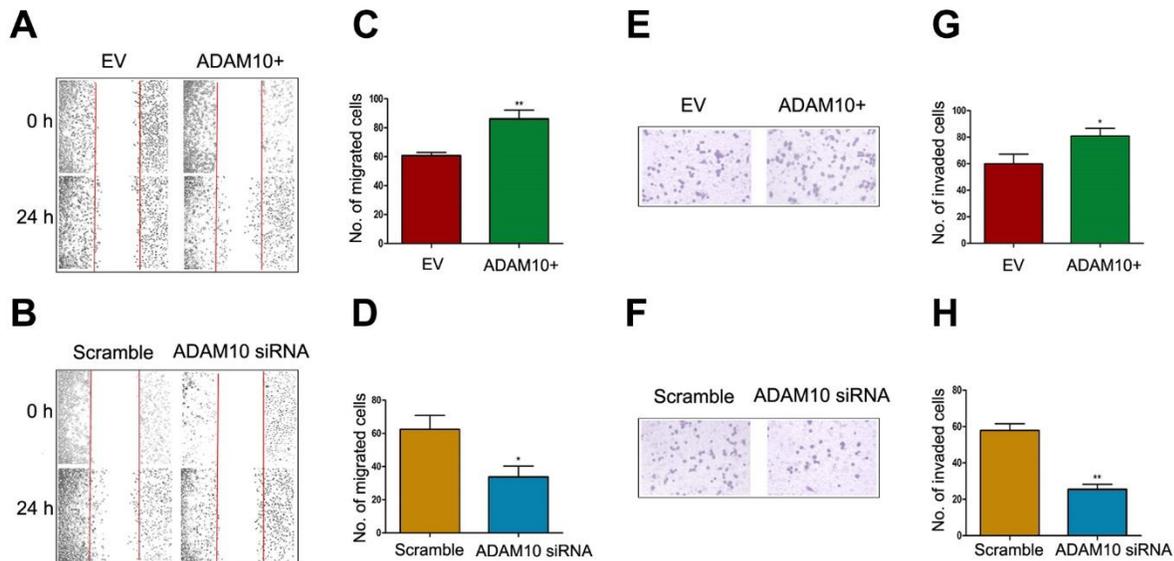


Fig. 2: Effect of ADAM10 expressions in PANC1 cells migration and invasion properties. After transfection (A) and (E) with pcDNA 3.1(+)/ADAM10 plasmid (ADAM10+) or an EV or (B) and (F) with a scramble siRNA or with siRNA targeting ADAM10 the cells were grown for 48 h. An *in vitro* (A-D) wound healing and (E-H) Transwell assays were conducted to evaluate cell migration and invasion, respectively. Data represented the mean of three independent experiments \pm SEM. *, $P < 0.05$, Student's t test

Impact of ADAM10 on metastatic and thrombus-associated genes

The expressions of metastatic and thrombus-associated markers TF, PSGL-1 and CTSG were firstly assessed by immunofluorescence. In the prepared cellular clones a moderate to strong surface protein expression of markers was detected in ADAM10 over-expressing cells while this was restricted or absent to the membrane when the cells were treated with siRNA targeting ADAM10 (Fig. 3).

To confirm the effect of ADAM10 expression on the regulation of mRNA and protein levels of TF,

PSGL-1 and CTSG, qRT-PCR and Western blot analysis were carried out. In ADAM10 over-expressing cells, the expression of these genes was coupled with the expression of ADAM10 at mRNA and protein levels when compared to EV clones (Fig. 4A-C). Similarly, knockdown of ADAM10 expression significantly decreased TF, PSGL-1 and CTSG expression levels compared to control cells, transfected with scrambled siRNA (Fig. 4D-F).

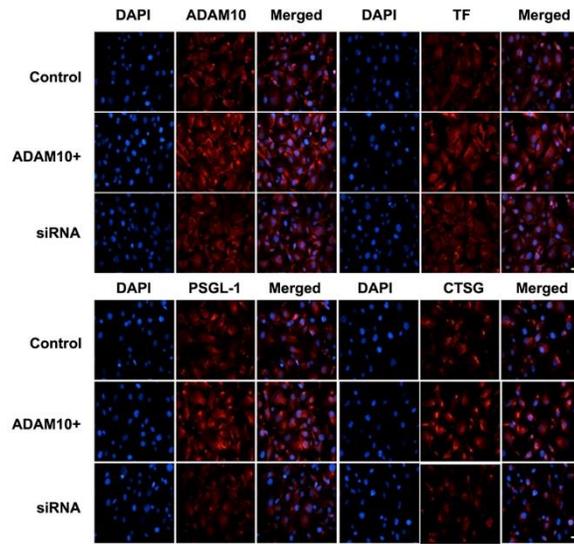


Fig. 3: Immunofluorescence staining of ADAM10 expression and its effect on metastatic and thrombus-associated genes in PANC1 cells. The control group or transfected with pcDNA 3.1(+)/ADAM10 plasmid (ADAM10⁺) or with ADAM10 siRNA as indicated. (Magnification, ×200). Bar=20 μm

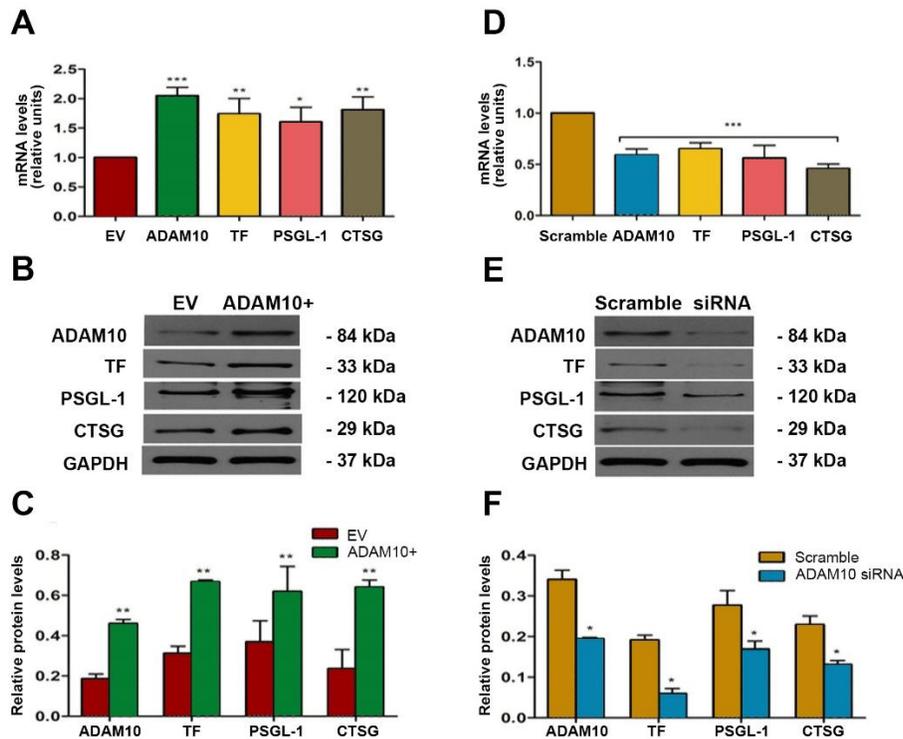


Fig. 4: Effect of ADAM10 expressions on the thrombus-associated genes in PANC1 cell lines. (A) effects of ADAM10 overexpression on *TF*, *CTSG* and *PSGL-1* mRNA levels, (B) effect of ADAM10 overexpression on protein levels (C) quantification of data in B. (D) effect of ADAM10 silencing on *TF*, *CTSG* and *PSGL-1* mRNA levels (E) effects of ADAM10 silencing on protein levels and (F) quantification of data presented in E. Graph bars represent the mean of three independent experiments ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, Student's *t* test

MUC1 and ADAM10 are dependent regulatory processes

We developed PANC1 clones expressing MUC1 and PANC1 clones silencing MUC1 expression using recombinant pcDNA 3.1(+)/MUC1 vector and siRNA strategies (si1.1, si1.2 and si1.3 targeting MUC1), respectively. When the cells treated with recombinant pcDNA 3.1(+)/MUC1 similar

impact on ADAM10 mRNA (Fig. 5A) and protein (Fig. 5B and C) expression levels was obtained. However, the siRNA transfected cells showed a significant reduction in ADAM10 mRNA (Fig. 5D) and protein expression levels (Fig. 5E and F) when compared to scramble siRNA transfected cells.

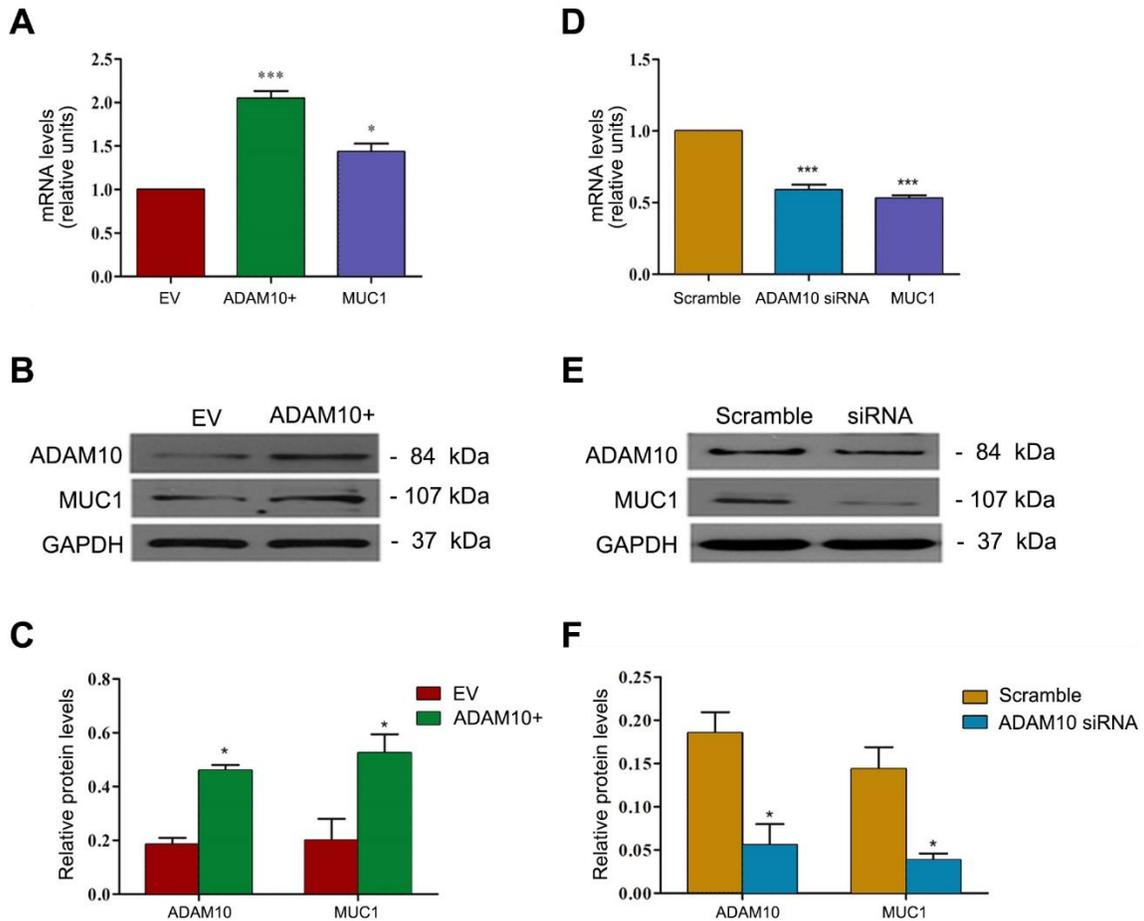


Fig. 5: Effect of ADAM10 expressions on MUC1 mRNA and protein levels in PANC1 cells. (A) effects of ADAM10 overexpression on MUC1 mRNA levels, (B) effect of ADAM10 overexpression on MUC1 protein levels (C) quantification of data in B. (D) effect of ADAM10 silencing on MUC1 mRNA levels (E) effects of ADAM10 silencing on protein levels and (F) quantification of data presented in E. Graph bars represent the mean of three independent experiments \pm SEM. *, $P < 0.05$; ***, $P < 0.001$, Student's t test

ADAM10 regulates tumor growth, metastatic properties and thrombus formation in vivo

In order to confirm that ADAM10 expression affect tumor cells behavior in vivo, NODSCID

mice were subcutaneously injected with the prepared clones of ADAM10 transfected cells. The results showed that when the mice treated with PANC1 cell clones, transfected with pcDNA

3.1(+)/ADAM10, significant increase in tumor volume was observed, compared to mice treated with EV clones ($P<0.05$). The relative tumor volume was $230 \pm 43.33 \text{ mm}^3$ in mice that treated with ADAM10 over-expressing cells while in EV

clones, the tumor volume was $115 \pm 20.12 \text{ mm}^3$ ($P<0.05$; Fig. 6A, C and E). Furthermore, down-regulation of ADAM10 reduced the tumor cells progression *in vivo* (Fig. 6B, D and F).

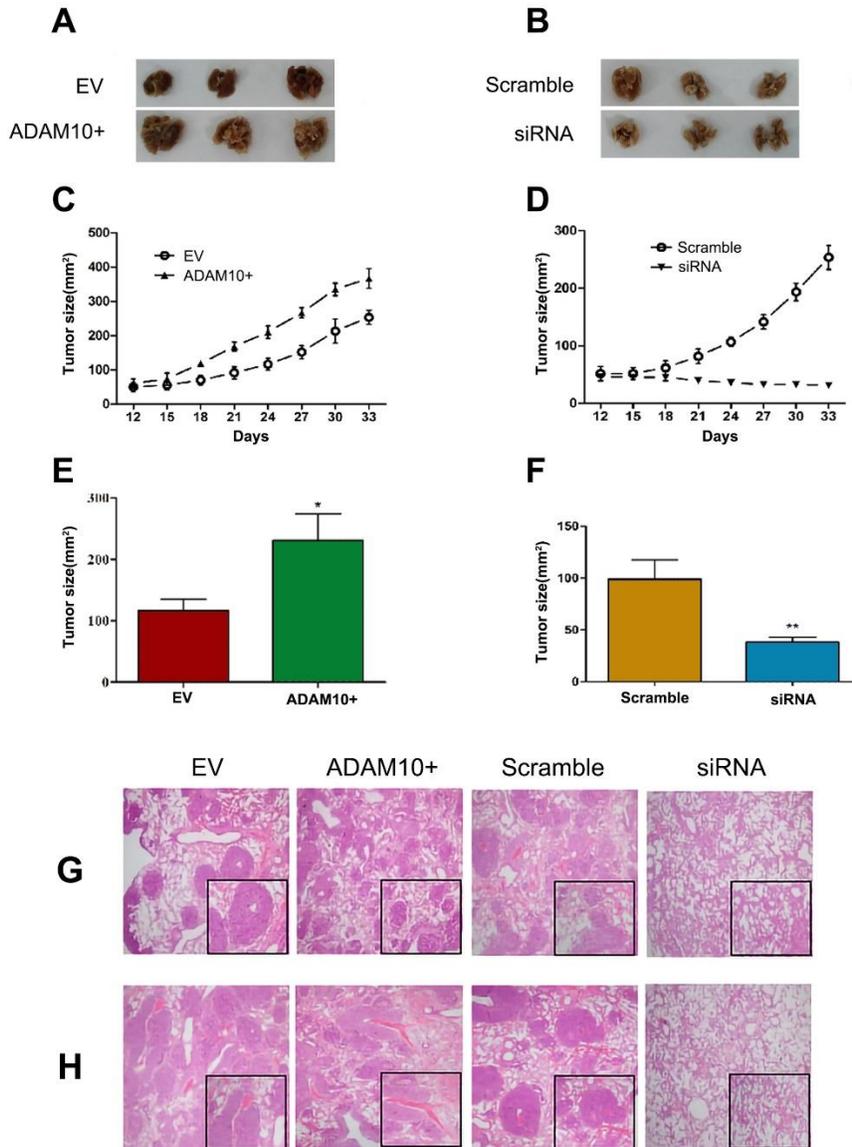


Fig. 6: Impact of ADAM10 expressions on tumor growth and size in pancreatic animal models. PANC1 cells were injected subcutaneously in 4 groups of the NODSCID mice by the following treatments: (A) recombinant pcDNA 3.1(+)/ADAM10 plasmid or an EV or (B) siRNA targeting ADAM10 or with non targeting scrambled siRNA. (C) and (D) measurements and presentation of tumor size (means \pm SEM) on the indicated days. (E and F) one-way ANOVA indicated that the change in tumor size, compared with the control groups, over time significantly differed in the treatment groups. Detection of lung metastasis (G) and (H) thrombus formation of H&E staining sections are displayed. Magnification: $\times 40$

The hematoxylin and eosin (H&E) staining was used to confirm the histological features of metastatic tumors. The proportion of distant metastases was increased when the mice treated with the ADAM10 over-expressing cells. Metastases were predominantly in mice lung whereas in case of ADAM10 silencing, no metastatic lesions were identified (Fig. 6G). In addition to its role in metastasis development, our results established that the generated PANC1 clones expressing ADAM10 was apparently lead to thrombus formation in distant sites, particularly in lungs. However, in PANC1 clones where ADAM10 expression was silenced with siRNA there was no detectable thrombosis in lungs (Fig. 6H).

Discussion

A lot is known about the role of ADAM10 in functional biology, but relatively little is known regarding the detailed mechanisms for their effect on metabolic activity in cancer cells. ADAM10 overexpression has been detected in different adenocarcinoma cells originated from different tissues. However, there was no study examined the direct effect of ADAM10 expression on thrombus-specific genes (*TF*, *CTSG* and *PSGL-1*) of metastatic and cancer-associated thrombosis in pancreatic cancer cell levels.

In this study, the expression level of ADAM10 was investigated in PANC1 to identify whether ADAM10 is responsible for the abnormal metabolic activity of these cancer cells, considering its impact on metastatic and thrombus-associated genes. We constructed different clones of PANC1 cancer cells that expressing and silencing ADAM10 using recombinant pcDNA 3.1(+)/ADAM10 vector and siRNA strategies. Furthermore, the SDS-PAGE detected the prodomain form of the ADAM10 (84 kDa) on PANC1 cancer cell lines. Then the biological function of ADAM10 prodomain in tumor cells metabolic behavior and its impact on metastatic and thrombus-associated genes was examined. The ADAM10 prodomain has been involved in proper folding of the enzyme and acts as an in-

tramolecular inhibitor on sheddase activities. An active form of ADAM10 was tumor specific in both mouse models and humans (7). Interestingly, their finding reflected considerable attention for the inhibition of active ADAM10 as a novel therapy.

Consistent with our results, Matthias et al (8), demonstrated that there was no significant effect on cell apoptosis of pancreatic cancer cells after ADAM10 silencing, however ADAM10 over expression significantly affect cells proliferation. Although cell migration and metastasis are primary features of aggressive tumors, ADAM10 is known to be implicated in the shedding of numerous migration-promoting and metastatic molecules, such as IL-6 receptor, TNF- α (9), CD44 and CD40L(6), which direct different processes such as tune of immune cells and their recruitments to transmigrate, recognize and remove tumor cells. Our results showed that overexpression of ADAM10 induces migration and invasion of PANC1 cells, and ADAM10 silencing was shown to disrupt these properties. The high metastatic potential of PANC1 cells expressing ADAM10 underscores the invasive properties, which influences tumor growth and largely determines metastatic potential. Indeed, the presence of metastasis is thought to be responsible for the aggressive thrombotic behaviors. A broad range of ADAM10 mRNA expression levels was observed in PANC1, which may be associated with the potential prognostic sign of pancreatic adenocarcinoma. Our finding is consistent with the particular thrombosis rates in these cancer types. 28.3% of pancreatic cancer patients were able to develop venous thromboembolism within one year of tumor metastasis, contrasted with rates of 7.4%, 5.7%, and 0.4% among lung, colon, and breast cancer patients (10).

Another explanation of metastasis and thrombogenicity mechanism may be that in pancreatic adenocarcinoma cells an increased expression of ADAM10 induces the molecular metastatic and cancer-associated markers *TF*, *CTSG*, *PSGL-1* and *MUC1* expressions. Interaction of P-selectin with its receptor *PSGL-1* leads to a procoagulant

state by mediating the transfer of primary initiator of coagulation, TF to platelets (9). In this study, the expressed TF and PSGL-1 in pancreatic cancer cells PANC1 may induce thrombus formation and metastasis. Beside TF and PSGL-1 expressions, both CTSG and MUC1 were expressed on PANC1 cells and they have ability to generate metastasis and microthrombi by different mechanisms. PSGL-1 and carcinoma mucins can interact with P-selectin of the platelets, depending on the release of neutrophil CTSG, the mediator of tissue injury, thrombus formation and inflammation (11). Together with our finding of a direct relationship between ADAM10 expression and the regulation of metastatic and thrombus-associated genes, they strengthen our hypothesis that ADAM10 plays a crucial role in various mechanisms underlying metastasis and thrombus induction.

Since MUC1 is a substrate of γ -secretase, several possibilities exist regarding its association with ADAM10 expression. Previous study demonstrated that MUC1 is involved in renal cancer cells development, and ADAM10 sheddases is necessary for invasiveness. This study confirmed that ADAM10 and MUC1 are developmentally regulated and aberrantly expressed on PANC1 cells by a dependent mechanism.

To our knowledge, this is the initial study to demonstrate that silencing of ADAM10 impaired TF, CTSG, PSGL-1 and MUC1 suggesting that ADAM10 activity is responsible for the overexpression of these receptors under pathological conditions. Our results provide evidence that ADAM10 silencing is a regulatory mechanism that controls the overexpression of TF, CTSG, PSGL-1 and MUC1, which are potentially associated with thrombus formation and metastatic potentials in cancer patients. Besides, ADAM10 expression significantly regulates the migration and invasion capacity of pancreatic cancer cells in wound healing and Transwell assays.

Conclusion

Based on ADAM10 expression, the cloned pancreatic cancer cells dramatically control tumor

cell growth, progression and thrombus formation when injected into mice model. Therefore, regulatory expression of ADAM10 might effectively pave the way for a more potent anti-cancer associated thrombosis in certain contexts and could regulate cell invasion and migration by disabling the abnormal metabolic behavior of cancer cells. There is a growing evidence supporting that ADAM10 activity might participate in the progression of cancer metastasis and thrombosis.

Ethics Journalism considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors

Acknowledgements

This research was funded by the National Natural Science Foundation of China (Nos. 81760093, 51803067 and 21774039), China Postdoctoral Science Foundation (General Program, Nos. 2018M630866, 2015M580640).

Conflicts of Interest

The authors declare that there is no conflict of interest.

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