Oncogenic KRAS Targets MUC16/CA125 in Pancreatic Ductal Adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with the 5-year survival rate less than 6%. Previous results indicated that serum levels of CA125 (encoded by MUC16) could be used to predict which groups of pancreatic cancer patients may benefit from surgery. However, the underlying mechanism remains elusive. Herein, using the Cancer Genome Atlas and clinicopathologic data obtained from our center, we demonstrate that high CA125 serum levels and expression levels of MUC16 are predictive of poor prognosis. MUC16 is also validated as a downstream target of KRAS, and their expression strongly correlated with each other in vitro and in vivo. Mechanistically, the KRAS/ERK axis induced upregulation of MUC16 and shedding of CA125 via its effector c-Myc in SW1990 and PANC-1 pancreatic cancer cells. Notably, proto-oncogene c-Myc could bind to the promoter of MUC16 and transcriptionally activate its expression. Taken together, these data establish CA125 as a prognostic marker for pancreatic cancer, and mechanistic studies uncovered the KRAS/c-Myc axis as a driving factor for upregulation of MUC16.

Implications: The current study uncovers the contribution of oncogenic KRAS to serum marker CA125 production through a mechanism that involves the ERK/c-Myc axis. Mol Cancer Res; 15(2), 201–12. ©2016 AACR.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) makes up the 90% of all pancreatic malignancies and is characterized by poor overall survival. Although significant progress has been made in diagnosis and treatment, PDAC is still a devastating disease with extremely high lethal rate, which is equal to the incidence of the disease (1-3). Thus, there is an urgent need for developing strategies in prediction of resectability, assessing the effect of chemotherapy and radiotherapy (4). Although medical image examinations like CT and ultrasound may provide much important information for tumor, it is still not sufficient to obtain complete data preoperatively. The detection of serum tumor markers is routinely used in a variety of tumor patients, because it involves noninvasive tests that facilitate the early detection, prediction of outcomes, and assessment of response to therapy (5).

CA19-9 is a specific tumor marker of digestive system tumors, especially pancreatic cancer, and is widely used in the diagnosis and monitoring of pancreatic cancer (6). It reflects the tumor burden and activity, and has predictive role for postoperative outcomes (7-9). CA125 was firstly detected in ovarian cancer and had been reported to be positive in 80% of ovarian epithelial tumor patients (10). In addition, the positive observation of CA125 is also seen in breast cancer, pancreatic cancer, gastric cancer, and some other tumors (11). Carcinoembryonic antigen (CEA) is a nonspecific tumor marker and is commonly used for detection of colorectal cancer (12). Moreover, CEA could also reflect the existence of a variety of tumors, such as pancreatic cancer, lung cancer, and gastric cancer (13, 14). Our previous studies reported that by combination use of CA19-9 with serum markers of CA125 and CEA, we could predict postoperative outcomes, and patients with baseline CEA+/CA125+/CA19-9 ≥ 1,000 U/mL were prone to larger tumors, lymph node metastases, and high tumor-node-metastasis stage (15). Thus, understanding the underlying mechanisms for the expression of these tumor markers will help to uncover novel strategies for the prediction and treatment for pancreatic cancer. Our most recent study demonstrated that CA125 could compensate CA19-9 in diagnosis and prognosis of Lewis-negative patients with pancreatic cancer, and received much attention for research into its underlying regulatory mechanism.

Circulating CA125 was the cleaved product of MUC16 on the cell surface, the largest membrane glycoprotein known. MUC16 is believed to play important roles not only in normal contexts such as reproduction and proliferation, but also in pathologic states including cancer and mucosal infections. It exerts its function in cancer by many mechanisms such as migration, invasion, cell cycle control and metabolism reprogramming (16, 17). Since its discovery, CA125 is usually used as a tumor marker, and many efforts have been given to its contribution to tumor malignancies,
yet little is known about its regulation. The importance of MUC16/CA125 in pancreatic cancer diagnosis and progression demands an urgent need for research on the expression regulation of this molecule.

The activating point mutation of Kras oncogene on codon-12 of exon-2 induces replacement of the GGT sequence (encoding for glycine) by the GAT sequence (aspartic acid–G12D–C35 C>A), which remains the major event in pancreatic cancer (18). It is found in approximately 95% of pancreatic intraepithelial neoplasias (PanIN), which is considered to be the initiating genetic event for PDAC progression (19, 20). The activation of KrasG12D inhibits intrinsic guanosine triphosphatase (GTPase) activity of RAS and permanently activates downstream signaling pathways. Thus, the signaling networks engaged by oncogenic KrasG12D are highly complex and characterized by the activation of a series of effectors leading to proliferation, progression, and changes in stroma interaction, immune system, and metabolic state (21, 22). However, the contribution of Kras to serum tumor markers production has seldom been discussed. Based on the contribution of Kras to malignant behavior maintenance of pancreatic cancer and the critical role of CA125 in cancer diagnosis and prognosis, we sought to uncover the correlation and underlying mechanism between Kras and MUC16/CA125 in the present study. In depth, mechanism analysis of this correlation may present novel opportunities for patient stratification, personalized therapies, and therapeutic targets.

Materials and Methods

Tissue specimens

The PDAC tissue microarrays used in this study were obtained from 110 patients diagnosed with pancreatic cancer at Fudan University Shanghai Cancer Center (FUSCC). Prior patient consent and approval from the Institutional Research Ethics Committee were obtained. Strict pathologic diagnoses and postoperative follow-ups were performed for all patients. Animal tissue slides derived from P48-Cre;KrasG12D;Ink4a/ArfF/F mice were generously provided by Professor Paul J. Chiao from MD Anderson Cancer Center.

Immunohistochemical staining

Immunohistochemical staining of paraffin-embedded tissues with antibodies for MUC16, phosphorylation of ERK (p-ERK), c-Myc, and FBW7 were performed and scored to determine the proteins expression according to standard procedures described previously (23).

The Cancer Genome Atlas dataset analysis

The Cancer Genome Atlas (TCGA)-Pancreatic adenocarcinoma (PAAD) on RNA expression (Level 3) of pancreatic cancer patients in terms of RNA-seq by expectation-maximization was downloaded from the Cancer Genomics Brower of the University of California, Santa Cruz (UCSC; https://genome-cancer.ucsc.edu/). In total, 160 primary pancreatic cancer samples from patients with detailed expression data were chosen from the updated TCGA database according to parameters mentioned.

Cell culture

The human pancreatic cancer cell lines PANC-1, MiaPaCa-2, CFPAC-1, Capan-1, BxPC-3, and SW1990 were obtained from the ATCC and cultured according to standard ATCC protocols. The cell lines have passed the conventional tests of cell line quality control and have DNA profiling of short tandem repeats that are consistent with those reported by the ATCC (Cell Bank & Type Culture Collection, Chinese Academy of Sciences). iKras cell lines, which express KrasG12D upon doxycycline (doxy) treatment, and human pancreatic nonmalignant epithelial HPNE cells were generously provided by Professor Paul J. Chiao. Panc-1 and SW1990 cell lines with FBW7 overexpression were generated previously (24).

Protein extraction and Western blot analysis

Cells were washed twice with ice-cold PBS and lysed in RIPA buffer for 10 minutes. Cell debris was removed by centrifugation at 12,000 rpm for 20 minutes at 4° C. Note that 20 µg total protein lysate was subjected to electrophoresis in denaturing 10% SDS-polyacrylamide gel, and then transferred to a membrane for subsequent blotting with specific antibodies. β-Actin and Kras antibodies were purchased from Proteintech. Antibodies against p44/42 MAPK (ERK1/2), phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204), and p44 MAP kinase (ERK1) were purchased from Cell Signaling Technology. c-Myc antibody was produced by Abcam company.

RNA isolation and quantitative real-time PCR

Total RNA was extracted by using TRIzol reagent (Invitrogen). To obtain cDNA, the Takara PrimeScript RT reagent Kit was used for reverse transcription. Expression status of candidate genes and β-actin were determined by quantitative real-time PCR using an ABI 7900HT Real-Time PCR system (Applied Biosystems). All reactions were run in triplicate. Primer sequences are listed as Supplementary Table S1.

Flow cytometry analysis

Surface expression of MUC16 on cells was assessed by single-color immunofluorescence and flow cytometry using primary anti-MUC16 mAb (X306) with appropriate FITC-conjugated secondary antibodies. Background levels were determined by incubating cell with properly matched FITC-conjugated isotype control antibodies.

Transfection of siRNA

All siRNAs were purchased from Biotrend company and transfected by using Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions. The sequences of siRNA oligos used in this study were as listed in Supplementary Table S2.

Transwell migration assay and invasion assay

A 24-well Transwell chamber with an 8-µm-pore PET membrane (BD Biosciences) was used to conduct the migration and invasion assays. The lower chamber was filled with 800 µl media containing 10% FBS. Subsequently, approximately 6 × 104 cells were seeded in 200 µl medium without serum in the top chamber for migration assay, and 10 × 104 cells were seeded into top chamber with a Matrigel-coated membrane (BD) for invasion assays. The cells were allowed to migrate at 37°C with 5% CO2 over 24 hours. After removal of the non-migrating or noninvasive cells, the remaining cells were washed, fixed, and stained with crystal violet. We counted the number of migrating and invading cells in six fields randomly selected at ×100 magnification. Experiments were performed at least in triplicate.
Measurement of CA125 in cell culture supernatants
CA125 levels in cell culture supernatants were quantitatively measured using Human CA125 ELISA Kit SimpleStep (Abcam), according to the manufacturer’s instruction. Briefly, the supernatants were added into the Pre-Coated 96 Well Microplate and then Antibody Cocktail was added into each well. The plate was incubated for 1 hour at room temperature on a plate shaker. Next, each well was washed with 1 × Wash Buffer PT to add TMB Substrate and incubated for 10 minutes. Lastly, at the endpoint, Stop Solution was added into each well to record the OD at 450 nm.

Analysis of promoter activity with dual luciferase assay
Promoters of mouse MUC16 and human MUC16 promoter regions, spanning from −2,500 to 200 of the transcription starting site, were cloned into pGL3-Basic vector. Cells were plated on 96-well culture plates and transfected with pGL3 constructs and Renilla luciferase expression vectors by using Lipofectamine 2000 (Invitrogen). Next, the cells were assayed for both firefly and Renilla luciferase activities using a dual-luciferase system (Promega), as described in the manufacturer’s protocol.

Chromatin immunoprecipitation assay
Chromatin immunoprecipitation (ChIP) was performed according to the instructions of the Magna ChIP A/G Chromatin Immunoprecipitation Kit (Merck Millipore Corporation). The nuclear DNA extracts were amplified using two pairs of primers (Supplementary Table S3) that spanned the MUC16 promoter region.

Statistical analyses
Statistical analyses were performed by SPSS software (version 17.0, IBM Corp.) using independent Student t test (two-tailed) or one-way ANOVA. Pearson correlation analysis was used to determine the correlation between Kras and MUC16 expression levels. The Fisher exact test was used to determine the correlation between MUC16 and clinicopathologic characteristics in the TCGA and FUSCC cohorts. Statistical significance was based on two-sided P values of <0.05.

Results
CA125/MUC16 expression positively correlated with Kras in pancreatic cancer
CA125 is a well-established marker in ovarian cancer. To assess its contribution to prognosis of pancreatic cancer, we examined the serum levels of CA125 in samples from FUSCC. In consistent with our previous reports, higher serum CA125 levels predicted poor prognosis (Fig. 1A). Next, we examined the expression status of MUC16 in the TCGA dataset and analyzed its contribution to prognosis. Clinical information regarding the samples is presented in Supplementary Table S4, and the level of MUC16 was an independent prognostic factor in pancreatic cancer by analysis of TCGA (Fig. 1B; Supplementary Table S5). To further validate this observation, we performed immunohistochemical staining to examine the MUC16 expression in PDAC tissue microarray from FUSCC as a validation cohort (Fig. 1C and Supplementary Table S6). In consistent with the results from the TCGA cohort, higher levels of MUC16 significantly correlated with poor survival (Fig. 1D and Supplementary Table S7). Kras expression and mutation is a decisive genetic event that associated with PDAC.

To assess the regulatory function of Kras in MUC16 expression and serum CA125 level, we examined the correlation of mutation status in Kras with serum level of CA125 in relevant patients. As observed, serum level of CA125 was significantly higher in Kras-mutant patients than that in Kras wild-type patients (Fig. 1E). By using the TCGA cohort, we analyzed the expression correlation between Kras with MUC16, as described, Kras expression strongly correlated with MUC16 expression (Fig. 1F). Moreover, in consistent with our observation concerning the correlation of mutation status in Kras with MUC16 expression in FUSCC cohort, we found in the TCGA cohort that MUC16 expression was significant higher in Kras-mutated patients in the TCGA dataset (Fig. 1G). To confirm the observation in the TCGA cohort and the FUSCC cohort, we examined the contribution of Kras mutation to MUC16 expression in vitro. Upon doxy induction in iKras cells, we observed that MUC16 expression significantly upregulated, along with KrasG12D induction (Fig. 2A and B). Given that MUC16 is a membrane bond macromolecule, we used the flow cytometry with MUC16 antibody to examine its level in the cell membrane in pancreatic cancer cell lines and HPNE cells (Fig. 2C). Through examination of the Kras mutation status of the cell lines used, we found that membrane-bound MUC16 levels were correlated to the metastatic potential and Kras status (SW1990 > CFPAC-1 > Capan-1 > Panc-1 > MiaPaca-2 > BxPC-3 > HPNE; Fig. 2D). Moreover, in samples from Kras-driven transgenic pancreatic cancer mice, we observed that MUC16 was higher in pancreatic cancer samples than that in normal control samples, further confirming our observation that Kras mutation contributed to MUC16 expression (Fig. 2E).

MUC16 was involved in the KrasG12D-mediated migration and invasion
MUC16 is a membrane protein that correlated with migration and invasion capacity of various cancer cells. To assess its role in Kras mutation–driven invasiveness, Kras expression significantly contributed the acquisition of migration and invasive capacity in iKras cells upon doxy treatment (Fig. 3A and B). Given that MUC16 expression significantly increased upon KrasG12D induction, we hypothesized that MUC16 was involved in Kras-induced metastasis. Next, we silenced MUC16 expression with siRNA oligos (Fig. 3C) and observed that silencing MUC16 inhibited Kras-induced migration (Fig. 3D and E). Furthermore, MUC16 inhibition also attenuated Kras-induced invasion capacity (Fig. 3F and G). These results suggested that mutant Kras-induced upregulation of MUC16 contributed to the migration and invasiveness in pancreatic cancer cells.

ERK activation is required for MUC16 expression in pancreatic cancer cells
In iKras cells, doxy induction could effectively induce the activation of ERK (Fig. 4A). Oncogenic Kras-induced ERK activation is vital for the Kras-induced malignant properties formation and maintenance. We speculated that ERK activation might account for MUC16 expression in pancreatic cancer cells. To test this hypothesis, we firstly inhibited ERK activation in iKras cells by treatment of the cells with UO126. As observed, UO126 treatment attenuated KrasG12D-induced expression of MUC16 (Fig. 4B). Next, in KrasG12D mutated SW1990 and Panc-1 cells, we observed that the expression of MUC16 decreased significantly in the presence of UO126 treatment, suggesting that ERK activation is required for MUC16 expression (Fig. 4C and D). Secreted
CA125 levels in SW1990 and PANC-1 cell culture medium also decreased when cells were treated with UO126, further supporting the role of ERK activation in MUC16 expression control (Fig. 4E).

Furthermore, we silenced ERK1 expression in SW1990 and PANC-1 cells to assess the regulatory role of ERK in MUC16 expression (Fig. 4F). In ERK1-silenced SW1990 and PANC-1 cells, the mRNA level of MUC16 decreased significantly (Fig. 4G). What is more, silencing ERK1 expression led to decrease of secreted CA125 levels in SW1990 and PANC-1 cells (Fig. 4H). Taken together, these results suggested that KrasG12D mutation–induced ERK activation is required for MUC16 expression and CA125 levels in SW1990 and PANC-1 cells.

c-Myc is the oncogenic KrasG12D effector that is responsible for MUC16 expression. c-Myc, a well-accepted Kras effector, as confirmed in our study (Fig. 5A), is a proto-oncogene and functions as a transcription factor that regulated the expression of many tumor-promoting genes. In iKras cells, JQ-1 treatment effectively lowered the level of c-Myc protein (Fig. 5B). Subsequently, we observed that JQ-1 effectively decreased KrasG12D-induced MUC16 expression, indicating that c-Myc might regulate MUC16 expression as a transcription factor (Fig. 5C). In KrasG12D-mutated SW1990 and PANC-1 cells, treatment with JQ-1 significantly decreased c-Myc expression, and the concomitant decrease in MUC16 expression (Fig. 5D and E). Furthermore, JQ-1 treatment significantly decreased CA125 level in cultured medium of SW1990 and PANC-1 cells (Fig. 5F). Next, we silenced c-Myc expression in SW1990 and PANC-1 cells to further explore its role in MUC16 expression (Fig. 5G). Silencing c-Myc significantly lowered MUC16 expression and shedding of CA125 in SW1990 and PANC-1 cells, indicating its promoting role in MUC16 expression (Fig. 5H and I). The similar results were also obtained in SW1990 and PANC-1 cells with c-Myc overexpression (Supplementary Fig. S1A and S1B).

Next, we analyzed the promoter region of MUC16 and found two putative c-Myc–binding E-box elements (Fig. 5J), and performed promoter luciferase assay to examine the role of c-Myc in MUC16 promoter activity regulation. As observed, JQ-1 treatment or manipulation of c-Myc expression altered MUC16 promoter activity (Fig. 5K). Furthermore, we performed ChIP assay to determine whether c-Myc occupied the E-boxes in the MUC16 promoter region. As shown, c-Myc enriched in the two E-boxes in the promoter region, further supporting its role in MUC16 expression.

Figure 1. MUC16 was involved in Kras mutation to predict a poor prognosis. A, High level of serum CA125 has a shorter overall survival of patients with pancreatic cancer. B, TCGA analysis for the association between tissue MUC16 and prognosis of pancreatic cancer. C, Immunohistochemical analysis for the tissue MUC16 expression in the FUSCC cohort. D, High level of tissue MUC16 predicts a poor prognosis to pancreatic cancer in the FUSCC cohort. E, The correlation between Kras status and serum CA125 level in patients with pancreatic cancer. F, TCGA analysis for the association between Kras and MUC16 expression in pancreatic cancer. G, TCGA analysis for the correlation between Kras status and MUC16 expression in pancreatic cancer.

Based on the above observations, we speculated that the ERK/c-Myc axis might be responsible for KrasG12D-induced MUC16 expression. We analyzed the promoter region in mouse MUC16 genome and found six putative c-Myc-binding E-box elements (Fig. 6A). We cloned the mouse MUC16 promoter into pGL3-basic vector for subsequent analysis. In iKras cells, UO126 or JQ-1 treatment significantly attenuated doxy-induced MUC16 promoter activity (Fig. 6B and C). Moreover, UO126 treatment or silencing ERK1 decreased the c-Myc protein level (Supplementary Fig. S2A and S2B), resulting in inhibiting the human MUC16 promoter activity in SW1990 and PANC-1 cells (Fig. 6D). These results suggested the contribution of ERK activation and c-Myc upregulation in MUC16 transcriptional expression. Furthermore, we observed that ERK inhibition by UO126 treatment or siRNA silencing decreased the occupancy of c-Myc in MUC16 promoter region (Fig. 6E and F; Supplementary Fig. S3A and S3B). To further validate the contribution of ERK/c-Myc axis in serum CA125 levels control, we examined the correlation between activation status of ERK and c-Myc level with serum CA125 level in pancreatic cancer patients samples. Results demonstrated that serum CA125 level was significantly higher in c-MycHigh groups of patients, although it was not statistically significant between p-ERKHigh and p-ERKLow groups (Fig. 6G and H).

FBW7 mediated ERK/c-Myc axis in regulation of MUC16 expression

FBW7 is an E3 ubiquitin ligase, and our previous study demonstrated that Kras/ERK/FBW7 signaling pathway participated in pancreatic cancer malignancies control including proliferation and metabolic reprogramming by targeting c-Myc. Previously, a high-throughput gene expression profiling array was performed and the microarray data were deposited in GEO under accession numbers GSE76443. Interestingly, we have found that MUC16 was one of these differentially expressed genes altered by FBW7 overexpression. Compared with control cells, the MUC16 expression in cells with FBW7 overexpression has decreased by 2-fold. Thus, it is naturally to ask whether FBW7 participated in MUC16 expression, and answers to this issue may provide intervention strategies in improvement of pancreatic cancer prognosis prediction and treatment. In SW1990 and PANC-1 cells with FBW7 overexpression, we observed a significant decrease in MUC16 expression (Fig. 7A). Moreover, the extracellular CA125 levels in FBW7-overexpressing SW1990 and PANC-1 cells were lower than the relative control cells (Fig. 7B). When FBW7 was silenced by siRNA, this down-regulatory phenomenon could be reversed (Supplementary Fig. S4A and S4B). To further test this hypothesis, we examined the correlation of serum CA125 level with FBW7 expression in pancreatic cancer patients. As illustrated, serum CA125 levels were higher in patients with lower FBW7 expression, further supporting the negative correlation between FBW7 and MUC16 expressions (Fig. 7C).
Next, we introduced FBW7 mutants to examine its role in MUC16 expression. As shown, introduction of wild-type FBW7 and its constitutively active mutant FBW7 T205A significantly decreased MUC16 promoter activity, whereas silencing FBW7 expression or exogenous introduction of dominant FBW7R465H mutant, a dominant-negative mutant that lost interaction with c-Myc, dramatically increased MUC16 promoter activity (Fig. 7D). Next, we assessed the role of FBW7 and the relative mutants in MUC16 expression and extracellular CA125 levels in SW1990 and PANC-1 cells. In consistent with the promoter activity assay results, introduction of FBW7 or the constitutively active mutant FBW7 T205A significantly decreased MUC16 expression and extracellular CA125 concentration in SW1990 and PANC-1 cells (Fig. 7E and F). On the contrary, exogenous expression of FBW7R465H dominant-negative mutant increased MUC16 expression and extracellular CA125 concentration in SW1990 and PANC-1 cells (Fig. 7E and F). To validate the in vitro results with cells lines, we assessed the contribution of FBW7 in combination with MUC16 in predicting prognosis. Patients with high CA125 and low FBW7 levels exhibited the worst overall survival, whereas CA125Low/FBW7High groups of patients shown a better survival (Fig. 7G).

In summary, we have identified expression and serum levels of MUC16/CA125 as a prognosis marker for overall survival by using the TCGA dataset cohort and FUSCC cohort. In depth, mechanism analysis uncovered that Kras G12D/ERK/FBW7/c-Myc axis is required for the transcriptional regulation of MUC16 expression in PDAC, and the existence of this axis has been further validated by using TCGA dataset analysis and clinicopathologic data obtained from FUSCC (Fig. 7H). These results will help to develop novel predictive and treatment targets for PDAC.

Discussion

In the present study, we demonstrated that MUC16/CA125 is a downstream target of mutant Kras G12D, and the expression levels of MUC16 and serum levels of CA125 positively correlated with Kras expression and mutation status. Further mechanism studies indicated that Kras-induced upregulation of CA125 depended on the activation of ERK kinase and the downstream effector protein...
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c-Myr. Moreover, FBW7 played a vital role in mediating Kras-induced MUC16 expression. Our studies provided novel predictive and treatment targets for pancreatic cancer.

The Kras gene encodes the protein of p21 RAS, which is a small GTPase that acts as a molecular switch by coupling cell membrane growth signals to intracellular signaling pathways and transcription factors to control multiple cellular processes (25, 26). Oncogenically mutated RAS proteins fail to cycle "off" from the active, GTP-bound state to the inactive GDP-bound state, and thereby accumulated in the constitutive "on" configuration (27). Kras is the most common oncogene that has been found to be mutated in pancreatic cancer. Oncogenic mutation of Kras resulted in constitutive activation of RAS proteins which permanently bound to GTP and led to subsequent activation of signaling pathways, such as the PI3K/AKT/mTOR or RAF/MEK/ERK signaling pathway. The activation of these pathways was involved in transformation, uncontrolled proliferation, invasion, and metastasis of pancreatic cancer cells (28). Detection of the Kras mutation status could be used to assess prognosis of pancreatic cancer (29–31). Moreover, the downstream targets of mutant Kras could also be used in the assessment of prognosis of pancreatic cancer (32). However, the combination of Kras mutation status with serum tumors marks in predicting prognosis, and the underlying mechanism has seldom been reported.

CA125 is a well-accepted tumor marker for many cancers. Its clinical role in pancreatic cancer received much attention recently (33, 34). Our previous study described a unique role for CA125 levels in diagnosis and treatment for pancreatic cancer. Higher CA125 levels specifically reflected the metastasis-associated tumor burden of pancreatic cancer in patients with advanced disease, as well as the presence of occult metastasis in patients with clinically localized tumors. Incorporation routine analysis of serum CA125 levels with CA19-9 and CEA in clinical examination both before and after pancreatic cancer treatments may help to improve therapeutic decisions and patient survival (15, 35). CA125 is the cleaved product of MUC16, a molecule with heavy molecular weights. The contribution of MUC16 to cancer malignancy has been studied in many cancer types. Silencing MUC16 studies in ovarian, breast, and pancreatic cancer cells impairs tumorigenic and metastatic properties (36–38). Moreover, MUC16 rendered antiapoptotic properties to cancer cells, leading to chemotherapy resistance (39). One recent study demonstrated that MUC16 promoted Warburg effect in pancreatic cancer by reprogramming glucose

Figure 4.
Activation of ERK increased the MUC16 expression in PDAC. A, Doxy treatment increased the phosphorylation level of ERK. B, Inducible activation of Kras increased the MUC16 expression, while this upregulation was inhibited by treatment with U0126, an inhibitor of MEK1/2. C, U0126 decreased the phosphorylation level of ERK in the SW1990 and PANC-1 cells. D, Inhibition of ERK kinase by U0126 decreased the expression of MUC16 in SW1990 and PANC-1 cells. E, Inhibition of ERK kinase decreased the shedding of CA125 in SW1990 and PANC-1 cells. F, ERK was decreased by siRNA-mediated silencing in SW1990 and PANC-1 cells. G, Knockdown of ERK decreased the expression of MUC16 in SW1990 and PANC-1 cells. H, The decreasing expression of ERK inhibited the shedding of CA125 in pancreatic cancer cells.
metabolism (40). Taken together, these observations identified MUC16/CA125 as an appropriate marker for prognosis and target for treatment in pancreatic cancer. However, the mechanism that accounts for MUC16/CA125 remains elusive and needs in-depth study. Answers to this question will help to develop novel predictive and treatment targets.

Figure 5.
Based on the decisive role of Kras mutation and the contribution of MUC16/CA125 in pancreatic cancer, we proposed that these two factors might have underlying correlations. Through analysis of the TCGA dataset, we observed that MUC16 expression was higher in Kras-mutant pancreatic cancer specimens than in wild-type Kras patients. The results were further confirmed in samples from our center, suggesting that MUC16 might be a mutant Kras target. Mounting evidence has pointed out c-Myc to be a Kras target (41). Moreover, we also reported that in pancreatic cancer, Kras mutation activated ERK, leading to destabilization of tumor suppressor FBW7, which ultimately led to c-Myc upregulation (24). These observations prompted us to consider MUC16 as a c-Myc downstream transcriptional target. Our further investigations supported this hypothesis and indicated that the ERK/FBW7/c-Myc axis was responsible for the upregulation of MUC16. The observations reported in the present study established a novel link between genetic variations with serum level of biomarkers in pancreatic cancer and provided underlying mechanism. Application of these findings will help to improve the efficacy of pancreatic cancer overall survival prediction and treatment. For example, CA19-9 is a tumor marker that reflects tumor burden and could be used to assess the efficacy of surgical resection, chemotherapy, and radiotherapy response in gastrointestinal tumors, such as pancreatic cancer and colorectal cancer (42, 43). The novel findings of the present study may point out further applications of utilizing Kras mutation and assessing CA125 level for measuring pancreatic cancer survival and response to traditional chemotherapy and radiotherapy. For example, endoscopic ultrasound-guided fine-needle aspiration biopsy is...
a useful tool in the diagnosis of pancreatic masses. Combination of genetic analysis of these samples with serum levels of tumor markers like CA19-9 could increase the sensitivity and specificity of diagnosis (44). However, no similar result has been reported by using Kras mutation status by conjugation with CA125 levels in improving sensitivity and specificity of diagnosis in pancreatic cancer, and this warrants further investigations. Moreover, even oncogenic Kras is considered as an undruggable target, and mounting efforts still have been put to develop strategies in targeting Kras and Kras-related signaling pathways in the hope of opening a therapeutic window for Kras-driven cancers (45). And once these trails become successful, the serum levels of CA125 could be used as an indicator for assessing efficacy.

In summary of the whole article, our studies uncovered a novel link between Kras mutation and serum marker of CA125, and provided the mechanistic explanations. These results will help to develop novel diagnostic, predictive, and treatment targets for pancreatic cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: Y. Qin, S. Ji, Q. Ni, X. Yu
Development of methodology: Y. Qin, B. Zhang, W. Xu, J. Xu

Figure 7.
FBW7 destabilized c-Myc protein to inhibit the MUC16 transcription. A, FBW7 overexpression significantly decreased the mRNA expression of MUC16 in SW1990 and PANC-1 cells. B, FBW7 overexpression decreased the shedding of CA125 in the PANC-1 and SW1990 cells. C, The serum levels of CA125 in the patients with high and low FBW7. D, Transfection of FBW7WT or its wild-type counterpart decreased the MUC16 promoter activity, whereas FBW7T205A and knockdown of FBW7 exerted little impact on MUC16 promoter activity compared with its wild-type counterpart. E, Transfection with wide type and mutant type of FBW7, T205A, and R465H into PANC-1 and SW1990 cells altered the MUC16 expression. F, The function and expression of FBW7 affect the shedding of CA125 in the PANC-1 and SW1990 cells. G, Kaplan-Meier analysis of the overall survival rate of patients with pancreatic cancer, according to FBW7 expression combined with the level of serum CA125. H, Proposed model of the mechanism of Kras-mediated regulation of metastasis via the c-Myc/MUC16 axis in pancreatic cancer.
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