DCAMKL-1 Regulates Epithelial–Mesenchymal Transition in Human Pancreatic Cells through a miR-200a–Dependent Mechanism

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Abstract

Pancreatic cancer is an exceptionally aggressive disease in great need of more effective therapeutic options. Epithelial–mesenchymal transition (EMT) plays a key role in cancer invasion and metastasis, and there is a gain of stem cell properties during EMT. Here we report increased expression of the putative pancreatic stem cell marker DCAMKL-1 in an established KRAS transgenic mouse model of pancreatic cancer and in human pancreatic adenocarcinoma. Colocalization of DCAMKL-1 with vimentin, a marker of mesenchymal lineage, along with 14-3-3σ was observed within premalignant PanIN lesions that arise in the mouse model. siRNA-mediated knockdown of DCAMKL-1 in human pancreatic cancer cells induced microRNA miR-200a, an EMT inhibitor, along with downregulation of EMT-associated transcription factors ZEB1, ZEB2, Snail, Slug, and Twist. Furthermore, DCAMKL-1 knockdown resulted in downregulation of c-Myc and KRAS through a let-7a microRNA-dependent mechanism, and downregulation of Notch-1 through a miR-144 microRNA-dependent mechanism. These findings illustrate direct regulatory links between DCAMKL-1, microRNAs, and EMT in pancreatic cancer. Moreover, they demonstrate a functional role for DCAMKL-1 in pancreatic cancer. Together, our results rationalize DCAMKL-1 as a therapeutic target for eradicating pancreatic cancers. Cancer Res; 71(6): 2328–38. ©2011 AACR.

Introduction

Pancreatic adenocarcinoma has the worst prognosis of any major malignancy with a 3% 5-year survival (1). Major obstacles in treating pancreatic cancer include extensive local tumor invasion and early metastasis. There is increasing evidence that a small subset of cells termed “cancer stem cells” (CSC) are capable of initiating and sustaining tumor growth (2). CSCs share unique properties with normal adult stem cells, including the ability to self-renew and differentiate. CSCs are often refractory to current standard chemotherapeutic agents and radiation therapies, as those treatment strategies are designed to eradicate actively cycling cells, but not slowly cycling CSCs. This results in tumor shrinkage but often fails to prevent tumor recurrence, due to the surviving CSCs ability to regenerate the tumor (3). Thus, novel therapies that specifically target the CSC population, either alone or in conjunction with current strategies may be more effective in obliterating solid tumors.

The existence of CSCs was first demonstrated in acute myelogenous leukemia (4) and subsequently verified in breast (5), pancreatic (3), and brain tumors (6–8). The CD133+ subpopulations from brain tumors could initiate clonally-derived neurospheres in vitro showing self-renewal, differentiation, and proliferative characteristics similar to normal brain stem cells (6–8). In a recent study, a subpopulation of CD44+CD24-ESA+ cells derived from primary human pancreatic adenocarcinomas CSCs (3) were implanted in immunocompromised mice and identified a subpopulation of cells with enhanced tumorigenic potential.

The 14-3-3σ gene was originally characterized as the human mammary epithelial-specific marker, HME1 (9). Besides its G2/M checkpoint functions, 14-3-3σ inhibits the proapoptotic proteins Bad and Bax (10, 11). 14-3-3σ is upregulated in lung cancer (12) and in head and neck carcinomas (13). Increased mRNA and protein expression of 14-3-3σ has been demonstrated in human pancreatic adenocarcinoma (14). Furthermore, several studies have demonstrated that 14-3-3σ contributes to the chemoresistance of pancreatic
cancer cells (15, 16). Therefore, strategies aimed at suppressing 14-3-3-σ expression and function may have a therapeutic benefit in pancreatic cancer.

MicroRNAs (miRNAs) are endogenous, approximately 22 nucleotide (nt) RNAs that play important regulatory roles at the posttranscriptional level in animals and plants by targeting miRNAs for cleavage or translational repression (17). miRNAs have emerged as important developmental regulators and control critical processes such as cell fate determination and cell death (17). There is increasing evidence that several miRNAs are mutated or poorly expressed in human cancers and may act as tumor suppressors or oncogenes (18, 19). Currently, the target genes of miRNAs are mainly identified by a combination of bioinformatic searches for potential miRNA recognition elements in the 3′-untranslated region (3′UTR) of the target gene. Subsequent experimental validation of predicted miRNA target interactions are conducted with luciferase reporter assays in cultured cells in vitro (17, 20).

We have recently demonstrated that DCAMKL-1, a microtubule-associated kinase expressed in postmitotic neurons, is a putative intestinal and pancreatic stem cell marker (21, 22). Furthermore, we have reported that DCAMKL-1, a protein expressed in both normal stem cells and in cancer, likely indicated miRNA target interactions are conducted with luciferase reporter assays in cultured cells (17, 20).

We have recently demonstrated that DCAMKL-1, a microtubule-associated kinase expressed in postmitotic neurons, is a putative intestinal and pancreatic stem cell marker (21, 22). Furthermore, we have reported that DCAMKL-1, a protein expressed in both normal stem cells and in cancer, likely promotes tumorigenesis through the regulation of pri-let-7a primary microRNA and c-Myc (23). Here we report that DCAMKL-1 is expressed in a subset of cells in human pancreatic tumors. We observed 14-3-3-σ in the cytoplasm and rarely in the nucleus of tumor epithelial cells in human pancreatic cancer patients. Interestingly, coexpression of DCAMKL-1 and 14-3-3-σ was observed in tumors. Moreover, we demonstrate DCAMKL-1 staining in the surface epithelium of pancreatic intraepithelial neoplasia (PanIN) type lesions and in the intervening stroma in human pancreatic adenocarcinoma. Knockdown of DCAMKL-1 in pancreatic cancer cells resulted in downregulation of Snail, Slug, and Twist and induction of microRNA miR-200a, which inhibits EMT. Furthermore, knockdown of DCAMKL-1 also resulted in downregulation of the protooncogenes c-Myc and KRAS via miR-144 miRNA-dependent mechanisms. These data taken together identify DCAMKL-1 as a novel pancreatic CSC marker that can potentially be targeted for pancreatic cancer eradication.

Materials and Methods

Tissue procurement

The human pancreatic adenocarcinoma (n = 10), pancreatic (n = 4), and normal appearing human pancreatic tissues (n = 3) were derived from patients undergoing a surgical resection of the pancreas at the University of Oklahoma Health Sciences Center and were made available to the policies and practices of the University’s IRB (protocol number 04586).

Cell culture

AsPC-1 and BxPC3 human pancreatic adenocarcinoma cell lines were purchased within 6 months of the experiments from the American Type Culture Collection (ATCC) and maintained as recommended. The cell lines were authenticated by ATCC.

Silencer RNA

DCAMKL-1 small interfering RNA (siRNA) (si-DCAMKL-1) sequence targeting the coding region of DCAMKL-1 (accession No. NM_004734) (GGGAGUGAGAACAAUCUACtt) and scrambled siRNAs (si-SCR) not matching any of the human genes were obtained (Ambion Inc) and transfected using siPORT NeoFX (Ambion Inc).

Immunohistochemistry, real-time reverse transcription-PCR analysis, miRNA analysis, and luciferase reporter gene assay

These analyses were carried out as previously described (23). Detailed descriptions are provided in the Supplementary section of Materials and Methods.

Scoring

Composite scoring for the immunostaining was performed by senior pathologist Dr. Stan Lightfoot, University of Oklahoma Health Sciences Center. Detailed descriptions are provided in the Supplementary section of Materials and Methods.

Stem/progenitor cell isolation from mouse pancreas

We isolated DCAMKL-1+ stem/progenitor cells from mouse pancreas as described earlier (22). Detailed descriptions are provided in the Supplementary section of Materials and Methods.

Results

DCAMKL-1 is expressed in the P48Cre-LSL-KRASG12D mouse pancreatic cancer model

The P48Cre-LSL-KRASG12D is a mouse model of pancreatic cancer that was initially developed by the Tyler Jacks laboratory (24). P48Cre-LSL-KRASG12D mouse model was originally developed on the 129V genetic background and later this model was backcrossed with C57BL/6 mice for more than 15 generations. When compared with 129V, the mutant mouse on the C57BL/6 genetic background develops more aggressive pancreatic lesions. These mice exhibit PanIN lesions after 10 weeks. Furthermore, these mice develop pancreatic adenocarcinomas with metastasis by 32 weeks (25). Pancreatic tissues from 5-month-old P48Cre-LSL-KRASG12D and their wild-type (WT) littermates were immunostained for DCAMKL-1. We found a marked increase in ductal immunoreactivity and a unique expansion of islet DCAMKL-1 in the P48Cre-LSL-KRASG12D pancreatic cancer mouse model that correlated with progressive neoplastic changes (Fig. 1A–D). Previously, using DNA microarrays, several groups have demonstrated increased 14-3-3-σ mRNA expression in pancreatic ductal adenocarcinoma compared with normal pancreas (14). Similarly, 14-3-3-σ protein nuclear localization has been described in pancreatic cancer (26). We found several DCAMKL-1+ cells within the PanIN lesions that also expressed nuclear 14-3-3-σ (Fig. 1E). Magnified images of this colocalization are shown in the Figure 1F and 1G. These data...
suggest that DCAMKL-1 is upregulated in pancreas of P48_Cre-LSL-KRAS<sup>G12D</sup> mouse and may play an important role in mutated KRAS-mediated tumorigenesis.

DCAMKL-1 in human pancreatic cancer

We examined DCAMKL-1 immunoreactivity in human pancreatic adenocarcinoma by immunohistochemical analysis. Samples were obtained from patients undergoing surgical resection of pancreatic cancer and pancreatitis provided by Dr. Russell Postier (Department of Surgery, The University of Oklahoma Health Sciences Center). Tumors demonstrated strong DCAMKL-1 protein localization. However, within the histologically normal appearing resection specimens, DCAMKL-1 was observed within islets but not in the intervening stromal cells or ducts (Fig. 2A top left; and Supplementary Fig. S1A). However, in chronic pancreatitis, we observed DCAMKL-1 in the islets, ducts and a few intervening stromal cells (Supplementary Fig. S1B-F). Within a neoplastic focus of the tumor resection specimen, however, intense spindle-shaped cytoplasmic staining of DCAMKL-1 was evident (Fig. 2A top right). DCAMKL-1 immunoreactivity in ductal epithelial cells within the tumor (Fig. 2A bottom left) and in intervening stromal elements was also observed (Fig. 2A bottom right).

14-3-3σ colocalizes with DCAMKL-1 in human pancreatic cancer

In normal appearing pancreatic tissue, we observed cytoplasmic staining for 14-3-3σ and DCAMKL-1 at the islet periphery, albeit in distinctly separate cells. We did not...
epithelial cells. We also observed cells with nuclear localized 14-3-3σ within tumor islet formations, similar to our observation in the mouse pancreatic cancer model. Among the nuclear 14-3-3σ expressing cells about 10% coexpressed DCAMKL-1 (Fig. 2C, left and right), suggesting that nuclear translocation of 14-3-3σ may occur in putative pancreatic CSCs. Furthermore, we observed an association of stromal DCAMKL-1 with the PanIN lesions in 40% of the tissue samples. Based on the composite scoring for DCAMKL-1 and 14-3-3σ immunostaining, we observed an increase staining of both the proteins in adenocarcinoma compared with normal (Supplementary Table S1). We also found DCAMKL-1 protein expression in human pancreatic PanIN lesions (Fig. 2D left), which increases in a stage-dependent manner compared with normal ductal epithelia (Supplementary Fig. 2A, B and C). In addition, we observed strong cytoplasmic 14-3-3σ and DCAMKL-1 coexisting within these lesions (Fig. 2D right). These data strongly support a role for 14-3-3σ and DCAMKL-1 in the progression of pancreatic cancer and colocalization of nuclear 14-3-3σ and DCAMKL-1 as a putative marker of pancreatic CSCs.

**DCAMKL-1 colocalizes with vimentin in the stroma of human pancreatic adenocarcinoma**

Initially, we observed DCAMKL-1+ staining in elongated cells in the surface epithelium of PanIN lesions (Fig. 3A, left). Further characterization of these cells using vimentin, as a marker of mesenchymal lineage, demonstrated that vimentin immunoreactive cells appeared morphologically similar to DCAMKL-1 positive cells (Fig. 3A, right). When double-labeled immunofluorescence was performed, colocalization of DCAMKL-1 and vimentin within the PanIN lesion was observed (Fig. 3B). As demonstrated earlier (Fig. 2A), fibrillar DCAMKL-1 staining was observed in approximately 40% of the stromal/mesenchymal compartment of the human pancreatic adenocarcinomas studied. The stromal nature of these cells was confirmed by co-staining with vimentin, where we observed 10% colocalization with DCAMKL-1 (Fig. 3C and D). These data taken together suggest that DCAMKL-1 may be involved in the desmoplastic reaction associated with human pancreatic cancer and may also play a role in EMT (27).

**DCAMKL-1 is involved in EMT**

EMT is a phenotypic conversion that facilitates organ morphogenesis and tissue remodeling in embryonic development and wound healing. A similar phenotypic conversion is also detected in fibrotic diseases and neoplasia, and is associated with disease progression and outcome (27, 28). Gene-profiling studies also suggest that mesenchymal gene profiles in tumors are predictive of poor outcome in colorectal, breast and ovarian cancers (2, 29).

Recent report suggests that the downregulation of several miRNAs (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) is an essential feature of EMT (30). Consequently, induction of these miRNAs results in inhibition of EMT (30–32). We have previously demonstrated that DCAMKL-1 negatively regulates tumor suppressor miRNA let-7a (23). To determine the potential role of DCAMKL-1 in EMT in pancreatic cancer,
we performed siRNA-mediated knockdown of DCAMKL-1 and evaluated miRNA expression of several candidate miRNAs known to play a role in EMT (31, 32). One such miRNA, miR-200a inhibits EMT by repressing the transcription factors ZEB1 and ZEB2 with subsequent rescue of E-cadherin (31, 32). Here we demonstrate that siRNA-mediated knockdown of DCAMKL-1 results in upregulation of pri-miR-200a (Fig. 4A) and downregulation of ZEB1 and ZEB2 with upregulation of E-cadherin (Fig. 4B) in the AsPC-1 human pancreatic cancer cell line.

The transcription factors Snail and Slug are key regulators of EMT and are expressed in pancreatic cancer but not in normal tissue, suggesting a role in the progression of human pancreatic tumors (33). In this report, we demonstrate that DCAMKL-1 colocalizes with Snail (Fig. 4C) and Slug (Fig. 4D) in human pancreatic cancer tissue. Furthermore, DCAMKL-1 knockdown results in the downregulation of Snail, Slug, and Twist (Fig. 4E) in AsPC-1 cells. These data taken together suggest that knockdown of DCAMKL-1 inhibits EMT via miR-200a-dependent mechanism in human pancreatic cancer.

**DCAMKL-1 regulates oncogenic c-Myc and KRAS**

We have recently demonstrated that DCAMKL-1 is a novel putative pancreatic stem/progenitor cell marker in the normal mouse pancreas (22). Furthermore, DCAMKL-1 negatively regulates let-7a miRNA (a tumor suppressor miRNA) in...
Figure 4. Knockdown of DCAMKL-1 inhibits EMT. A, DCAMKL-1 specific siRNA (siDCAMKL-1) decreases DCAMKL-1 mRNA expression (left), DCAMKL-1 protein expression (center) and increases expression of pri-miR-200a (right) compared with scrambled siRNA (siSCR)-treated or Control untreated AsPC-1 human pancreatic cancer cells. B, AsPC-1-siDCAMKL-1 cancer cells demonstrated decreased expression ZEB1 (left), ZEB2 (center) and rescues/upregulates E-cadherin (right). C, DCAMKL-1 (red) and Snail (green) in human pancreatic adenocarcinoma. Colocalization demonstrated in merged image and nuclei are stained blue with Hoechst dye (100×). D, DCAMKL-1 (red) and Slug (green) in human pancreatic adenocarcinoma. Colocalization demonstrated in merged image and nuclei are stained blue with Hoechst dye (100×). E, siRNA-mediated knockdown of DCAMKL-1 decreases Snail (left), Slug (center), and Twist (right) mRNA expression in AsPC-1 cancer cells. Insets in the images on the right in C and D are magnified images. For A, B, and E values given as mean ± SEM, and asterisks denote statistically significant differences (*P <0.01) compared with control.
normal mouse pancreas (Supplementary Fig. S3A and B) and human colorectal cancer cells (23). Moreover, let-7a negatively regulates several key oncogenes including c-Myc and KRAS in various solid tumors (23, 34, 35). To determine whether DCAMKL-1 regulates let-7a miRNA in pancreatic cancer cells, control, scrambled and DCAMKL-1 siRNA-treated human pancreatic cancer cell lines (AsPC-1 and BxPC3) were analyzed for pri-miRNA expression by real-time RT-PCR. Compared with control and siSCR-treated cells, there was a 3-fold increase in pri-let-7a miRNA in DCAMKL-1 siRNA-treated cells (Fig. 5A and Supplementary Fig. S4A and B). Thus, DCAMKL-1 negatively regulates pri-let-7a miRNA in human pancreatic cancer cells. To determine quantitatively the effect of siRNA-mediated knockdown of DCAMKL-1 on let-7a miRNA, we performed a luciferase reporter gene assay (23). AsPC-1 cells were transfected with a plasmid containing firefly luciferase gene with a complementary let-7a binding site in the 3’UTR. A dose-dependent reduction in luciferase activity was observed following the knockdown of DCAMKL-1 (Fig. 5B). These data taken together suggest that DCAMKL-1 may be a posttranscriptional regulator of let-7a miRNA downstream targets in pancreatic cancer.

Others and we demonstrated that c-Myc is a key downstream target of let-7a miRNA (23, 34). To demonstrate this in pancreatic cancer cell line, control, AsPC-1-siSCR and AsPC-1-siDCAMKL-1 cells were analyzed for c-Myc expression by...
DCAMKL-1 Knockdown Inhibits Pancreatic Cancer EMT

real-time RT-PCR. Compared with control and AsPC-1-siSCR cells, there was a significant ($P < 0.01$) 50% reduction of c-Myc mRNA expression in AsPC-1-siDCAMKL-1 cells (Fig. 5C). Similarly, a reduction in c-Myc protein was observed following the knockdown of DCAMKL-1 (Fig. 5C).

KRAS is a critical gene that is mutated in many cancers including pancreatic cancer and several studies have reported that up to 95% of pancreatic cancers contain KRAS mutations (36). KRAS is an another key downstream target of let-7a miRNA (35). Following knockdown of DCAMKL-1, we observed a 45% reduction in KRAS mRNA expression compared with control or AsPC-1-siSCR cells (Fig. 5D). To determine the mechanism of siRNA-mediated knockdown of DCAMKL-1 on KRAS, we transfected a KRAS-Luc reporter vector containing specific binding sites for let-7 family members within the 3’UTR of the firefly luciferase gene (similar to KRAS 3’UTR). A dose-dependent reduction in luciferase activity was observed following knockdown of DCAMKL-1 (Fig. 5E). Similar results were observed in the BxPC3 human pancreatic cancer cell line (data not shown). These data taken together demonstrate that DCAMKL-1 knockdown results in down-regulation of c-Myc and KRAS, 2 key mediators of tumorigenesis in pancreatic cancer.

DCAMKL-1 regulates Notch-1 in pancreatic cancer via miR-144

Notch signaling is frequently dysregulated in human malignancies (36, 37). Notch plays a key role in several cellular developmental pathways including proliferation and apoptosis (38). Upregulated expression of Notch receptors and their ligands has been described in cervical, lung, colon, head and neck, renal carcinoma, acute myeloid, Hodgkins lymphoma, large-cell lymphomas, and pancreatic cancer (39–43). Notch signaling is required for initiation and progression of pancreatic ductal adenocarcinoma (36). Furthermore, inhibition of Notch signaling using a γ-secretase inhibitor (MRK-003) completely inhibited tumor development in Pdx1-Cre; LSL-KRASG12D; p53lox/− mouse model of pancreatic neoplasia (36). Given the potential roles of Notch signaling in adult stem cell regulation and tumorigenesis (44), we investigated the effect of siRNA-mediated knockdown of DCAMKL-1 on Notch-1 in pancreatic cancer cells.

In this study, we observed a 50% reduction in Notch-1 mRNA in AsPC-1-siDCAMKL-1 cells compared with control AsPC-1 or AsPC-1-siSCR cells (Fig. 6A). Similar results were obtained in BxPC3 cells (Supplementary Fig. S4C). To determine the mechanism by which Notch-1 is inhibited, we first performed a computational/bioinformatics (www.microrna.org; A resource for microRNA targets and expression) analysis of the Notch-1 3’UTR. We found a predicted binding site for miR-144 in the Notch-1 3’UTR (at the 189th base pair; Fig. 6B).

To investigate the role of DCAMKL-1 in the regulation of miR-144 miRNA, control, scrambled, and DCAMKL-1 siRNA-treated AsPC-1 cells were analyzed for pri-miR-144 miRNA expression by real-time RT-PCR. Compared with control and AsPC-1-siSCR cells, there was a 2.5-fold increase in pri-miR-144 miRNA expression in AsPC-1-siDCAMKL-1 cells (Fig. 6C). These data suggest that DCAMKL-1 negatively regulates pri-miR-144 miRNA in human pancreatic cancer cells. Similarly, DCAMKL-1 was also found to negatively regulate pri-miR-144 in normal mouse pancreas (Supplementary Fig. S3C).

To evaluate these findings quantitatively, we performed a luciferase reporter gene assay using AsPC-1 cells that were transfected with a plasmid containing the firefly luciferase gene with a complementary miR-144 binding site in the 3’UTR. A dose-dependent reduction in luciferase activity was observed following DCAMKL-1 knockdown (Fig. 6D), indicating that DCAMKL-1 may be a posttranscriptional regulator of miR-144 miRNA downstream targets in pancreatic cancer. Taken together, these data strongly suggest that Notch-1 is a downstream target of miR-144 miRNA and that DCAMKL-1 regulates posttranscriptional control of Notch-1.

Discussion

Solid tumors are histologically heterogeneous and include tumor cells, stroma, inflammatory infiltrates, and vascular structures. The CSC hypothesis suggests that tumors are initiated and maintained by a minority subpopulation of cells within the tumor that have the capacity to self-renew and to generate more differentiated, rapidly proliferating, cells that make up the bulk of a tumor (2, 45).

The existence of CSCs has profound implications for cancer biology and therapy due to the likelihood that eradication of CSCs is the critical determinant in achieving a cure. Recent reports have demonstrated that breast and glioblastoma CSCs are radioresistant and may therefore contribute to treatment failures (46, 47). The cell surface marker CD133 is widely used for isolating CSCs from various cancers (48). In addition, a subpopulation of CD44+CD24−ESA+ cells was identified as putative pancreatic CSCs (3, 48). However, in general, most cell surface proteins used for isolation of CSCs serve as purification markers without functional implication (2, 48). Thus, it is critical to demonstrate that isolated cells from any particular cancer tissue have the functional characteristics of CSCs. Currently, this has been most convincingly demonstrated by serial transplantation in animal models (2).

We have previously demonstrated that DCAMKL-1 is upregulated in human colorectal cancers and siRNA-mediated knockdown of DCAMKL-1 results in tumor growth arrest via let-7a miRNA dependent manner (23). In this report, we provide evidence that DCAMKL-1 is upregulated in pancreatic cancer and may also identify pancreatic CSCs. Interestingly, we observed coexpression of DCAMKL-1 and 14-3-3 σ, an inhibitor of Bad proapoptotic activity, within human pancreatic adenocarcinomas (10, 11). Colocalization of 14-4-3 σ and DCAMKL-1 is significant as it may represent a target cell within tumors where 14-3-3 σ is transcriptionally activated. We also observed distinct DCAMKL-1 immunostaining in the intervening stroma between epithelial tumor elements, which coexpressed vimentin. These findings were indeed surprising in that we did not observe DCAMKL-1 in nonepithelial cells under basal conditions. We next evaluated ductal DCAMKL-1 within PanIN lesions. There we observed several elongated cells that also coexpressed vimentin and DCAMKL-1 suggesting that these cells are of mesenchymal origin. These findings
suggest that DCAMKL-1 expressing cells may be undergoing EMT (27). Desmoplasia, the appearance of fibrous, mesenchymal-like tissue in the peritumor stroma, is associated with poor clinical outcome (28). Indeed, gene-profiling studies suggest that mesenchymal gene profiles in tumors are predictive of poor clinical outcome (2). Myofibroblasts have long been thought to be derived from fibroblasts, but recent data has shown that a substantial proportion of these cells are derived from EMT and are associated with tumor progression (29).

We have previously demonstrated a functional role for DCAMKL-1 in the regulation of let-7a, a key tumor suppressor miRNA in many cancers including colorectal cancer. miRNAs are important regulators of mRNAs at the posttranscriptional level by targeting them for cleavage or translational repression (17). miRNAs have emerged as important developmental regulators and control critical processes such as cell fate determination and cell death (17). There is increasing evidence that several miRNAs are mutated or poorly expressed in human cancers and may act as tumor suppressors or oncogenes (18, 19). Here we report that DCAMKL-1 regulates miR-200a, let-7a, and miR-144 in the AsPC-1 pancreatic cancer cell line. Each of these miRNAs has been shown to play important
roles in several key aspects of tumor initiation and progression. For example, miR-200a inhibits EMT in several cancers by inhibiting transcription factors ZEB1 and ZEB2 (31, 32). Indeed, in this report, knockdown of DCAMKL-1 induces miR-200a resulting in downregulation of ZEB1, ZEB2, Snail, Slug, and Twist in pancreatic cancer cell lines. In addition let-7a, a tumor suppressor miRNA, has been shown to inhibit several key oncogenes. Following knockdown of DCAMKL-1, we observed a marked increase in let-7a, which resulted in downregulation of protooncogenes c-Myc and KRAS in pancreatic cancer cell lines using real-time RT-PCR and luciferase reporter assays. This is similar to our previous report demonstrating that DCAMKL-1 regulates c-Myc via let-7a miRNA in colorectal cancer cells (23). These data strongly support a direct regulatory role for DCAMKL-1 in cancer via miRNA-dependent mechanisms. DCAMKL-1 knockdown in AsPC-1 cells resulted in a marked decrease in Notch-1 mRNA (50%), which contains a putative predicted binding site for miR-144 in the 3’UTR. miR-144 is a regulator of embryonic-hemoglobin (E1), through targeting the 3’-UTR of Krüppel-like factor D gene and positively regulates erythroid differentiation in hematopoietic stem cells. To determine whether DCAMKL-1 regulates Notch-1 through a novel microRNA, we evaluated the expression of miR-144 in AsPC-1-siDCAMKL-1 cells. Here for the first time, we report that DCAMKL-1 negatively regulates Notch-1 via miR-144 dependent mechanism. These data taken together clearly demonstrate a multifunctional role for DCAMKL-1 in regulation of miRNAs that control important genes that contribute to key aspects of tumorigenesis (Fig. 7).

As recently reported, the induction of EMT in human mammary epithelial cells resulted in a "stem-cell-like" phenotype characterized by a CD44high and CD24low cell surface marker expression pattern. Furthermore, these cells formed mammospheres, colonies in soft agar and tumors in nude mice more aggressively than non-EMT induced cells. These studies demonstrate a direct link between the induction of EMT and the gain of stem-cell-like properties (49). These recent findings lend support to our hypothesis that EMT in the stem cell population may play a critical role in tumorigenesis. Further studies are needed to clearly define the role of DCAMKL-1 and other potential stem cell proteins (BMI-1 and LGR5) in cancer progression, metastasis, and EMT. Nevertheless, the studies presented here provide strong evidence that DCAMKL-1 may be an important target for therapy to eradicate pancreatic cancer and perhaps other solid tumors.

The Notch signaling pathway is frequently activated in pancreatic cancer (36). Inhibition of Notch signaling using a g-secretase inhibitor (MRK-003) completely blocked tumor development in Pdx1-Cre; LSL-KRASG12D; p53lox/+ mice (36). siRNA-mediated knockdown of DCAMKL-1 in human pancreatic cancer cell lines resulted in 50% reduction in Notch-1 mRNA. These data suggests that DCAMKL-1 disruption results in inhibition of the Notch-1 pathway thereby confirming its role as a potential target in anticancer strategies.

Disclosure of Potential Conflicts of Interest

C.W. Houchen and R. Ramanujam are cofounders of COARE Biotechnology, Inc. The other authors disclosed no potential conflicts of interest.

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Figure 7. Selective blockade of DCAMKL-1 results in inhibition of EMT and tumorigenesis in CSCs of pancreatic cancer. Inhibition of Notch signaling using a g-secretase inhibitor (MRK-003) completely blocked tumor development in Pdx1-Cre; LSL-KRASG12D; p53lox/+ mice (36). siRNA-mediated knockdown of DCAMKL-1 in human pancreatic cancer cell lines resulted in 50% reduction in Notch-1 mRNA. These data suggests that DCAMKL-1 disrupts EMT in several cancers.

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