PROTEIN KINASE C IOTA IS REQUIRED FOR PANCREATIC CANCER CELL TRANSFORMED GROWTH AND TUMORIGENESIS

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ABSTRACT: Pancreatic cancer is the fourth leading cause of cancer deaths in the United States with an overall 5-year survival rate of <5%. Pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer, is highly resistant to conventional chemotherapies. Therefore, there is a critical need for new molecular targets for pancreatic cancer therapeutics. Inactivation of the PTEN tumor suppressor gene occurs in >70% of PDAC. Oncogenic KRAS-driven tumors activate several oncogenic signaling pathways, including the atypical protein kinase C iota (PKCι) and PKCι is required for oncogenic Ras-mediated transformed growth in lung cancer and pancreatic cells. However, little is known about the role of PKCι in pancreatic cancer. In this study we evaluated the requirement for PKCι for the transformed growth and tumorigenicity of PDAC cells. Our results demonstrate that PKCι is significantly over-expressed in human pancreatic cancer and is required for PDAC cellular transformation in vitro and in vivo. Specifically, inhibition of PKCι expression blocks PDAC cell transformed growth in vitro and tumorigenicity in vivo. Analysis of PKCι downregulatory effects identifies Rac1(PIP2-dependent)90/Rho kinase signaling in PKCι-mediated transformed growth. Inhibition of PKCι expression in orthotopic pancreatic tumors also significantly reduces tumor angiogenesis and metastases. Taken together, our data demonstrate a requirement for PKCι in the transformed growth of pancreatic cancer cells and document a novel role for PKCι in pancreatic cancer cell metastasis and angiogenesis in vivo. These results strongly suggest that PKCι will be an effective target for pancreatic cancer therapy.

Figure 1: PKCι is highly expressed in human pancreatic cancer and PDAC cell lines.

A) qPCR analysis of PKCι mRNA expression in 26 matched human pancreatic tumor and adjacent non-tumor pancreas. mRNA abundance is normalized to 18S rRNA and mean ± SEM is plotted. B) Representative western blot analysis of PKCι in human pancreatic samples. C) qPCR analysis of PKCι mRNA expression in human pancreatic cancer cell lines. mRNA abundance is normalized to GAPDH mRNA and mean ± SEM is plotted. D) Immunohistochemical detection of PKCι expression in normal human pancreas (left, right) and human pancreatic cancer (bottom, right panel). E) qPCR analysis of PKCι mRNA expression in ten human pancreatic cancer cell lines (top). mRNA abundance is normalized to GAPDH mRNA and mean ± SEM is plotted. (Reprinted with permission from AACR 2008, #8931)

Figure 2: PKCι is not required for anchorage-dependent (transformed) growth of PDAC cells.

A) qPCR analysis of PKCι mRNA expression in A) Panc-1 and B) MiaPaCa-2 cells stably carrying either PKCι RNAi or control RNAi. Analysis was performed in triplicate and is representative of two independent experiments. C) Western blot analysis of PKCι protein in Panc-1 and B) MiaPaCa-2 cells stably carrying either PKCι RNAi or control RNAi. Immunoblotting was performed by MTT chromogenic assay.

Figure 3: PKCι is required for anchorage-independent growth of PDAC cells.

A) Soft agar colony formation of A) Panc-1 and B) MiaPaCa-2 cells stably carrying PKCι RNAi (PKCι#1 and PKCι#2) constructs. Mean ± SEM is plotted. B) Representative immunohistochemical detection of PKCι protein expression in Panc-1 cells stably carrying RNAi (NT or PKCι) and control vector (pBabe) or vector expressing constitutively active PKCι (PKCιCA). C) Re-expression of NT PKCι overcomes the inhibitory effect of PKCι RNAi on soft agar colony formation. ** significantly different than control (NT). D) qPCR analysis of PKCι and β-actin expression in Panc-1 NT versus Panc-1 PKCι RNAi, control vector and PKCιCA. Each sample was assayed for PKCι and β-actin expression in three independent experiments. E) qPCR analysis of PKCι expression in MiaPaCa-2 NT versus MiaPaCa-2 PKCι RNAi, control vector and PKCιCA. Each sample was assayed for PKCι expression in three independent experiments.

Figure 4: Constitutively active Rac1 recovers transformed growth of PKCι, RNAi PDAC cells.

A) Panc-1 cells stably expressing NT or PKCι RNAi were assayed for Rac1 activity. (Active) Rac1–GTP was precipitated from cell extracts with PAK-1 PBD agarose. Immunoblot analysis of precipitates and total cellular extracts were performed using an anti-Rac1 Ab. Mean ± SEM is plotted and represents at least two independent experiments. B) Rac1 activity in Panc-1 NT versus Panc-1 PKCι RNAi (PKCι#1 and PKCι#2). Analysis was performed using an anti-Rac1 Ab. Bottom panel, Quantitative, densitometric analysis of relative Rac1 activity (active Rac1/total Rac1). Mean of three independent experiments ± SEM is plotted. C) Immunohistochemical detection of PKCι expression in Panc-1 RNAi orthotopic pancreatic tumors. Equivalent amounts of protein from each tumor sample were analyzed. D) Representative immunohistochemical detection of CD31 staining. Bar=100µm. E) Percent of orthotopic Panc-1 NT and PKCι RNAi pancreatic tumors that metastasized to various organs. * Percent of orthotopic Panc-1 NT and PKCι RNAi pancreatic tumors that metastasized to various organs is plotted. ** significantly different than NT RNAi tumors.

Figure 5: Inhibition of PKCι blocks PDAC angiogenesis and metastasis.

A) Left, Immunohistochemical detection of CD31 staining. Right, Quantitative analysis of CD31 positive staining in Panc-1 tumors, calculated as the ratio of CD31 positive pixels to the sum of all pixels. Mean ± SEM is plotted. B) Representative immunohistochemical detection of VEGER and actin in Panc-1 NT and PKCι RNAi orthotopic pancreatic tumors. Equivalent amounts of protein from each tumor sample were assayed. C) Representative immunohistochemical detection of PKCι, phospho-ERK1/2 (Thr202/Tyr204), ERK1/2 (Thr202/Tyr204) and phospho-ERK1/2 (Thr202/Tyr204) in Panc-1 cells co-transfected with RNAi (PKCι#1 and PKCι#2) or vector expressing constitutively active Rac1 (caRac1) and control vector (LZRS). Top panel, (Active) Rac1–GTP was precipitated from cell extracts with PAK-1 PBD agarose. Immunoblot analysis of precipitates and total cellular extracts were performed using an anti-Rac1 Ab. Bottom panel, Quantitative, densitometric analysis of relative Rac1 activity (active Rac1/total Rac1). Mean ± SEM is plotted and represents at least two independent experiments. D) Re-expression of WT PKCι on soft agar colony formation. ** significantly different than control (NT). E) qPCR analysis of PKCι, β-actin mRNA expression in Panc-1 cells expressing NT, PKCι#1 and PKCι#2. Each sample was assayed for PKCι and β-actin expression in two independent experiments.

CONCLUSIONS:
PKCι expression is highly overexpressed in a high percent of primary pancreatic cancer tumors and highly induced in PDAC cell lines. PKCι is dispensable for adherence-dependent growth, but is required for transformed growth of pancreatic carcinoma cells in vitro. PKCι is downstream in the Rac1 signalling pathway involved in PKCι-dependent PDAC tumor cell proliferation in vivo. Inhibition of PKCι expression in PDAC cells reduces PDAC tumor angiogenesis and metastasis. Our results demonstrate a requirement for PKCι in transformed growth and oncogenic signaling in pancreatic cancer cells in vitro and in vivo. Together, these data strongly implicate PKCι as a candidate therapeutic target for the treatment of pancreatic cancer.

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