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Protein kinase C zeta plays an important role in the oncogenic phenotype of pancreatic cancer cells

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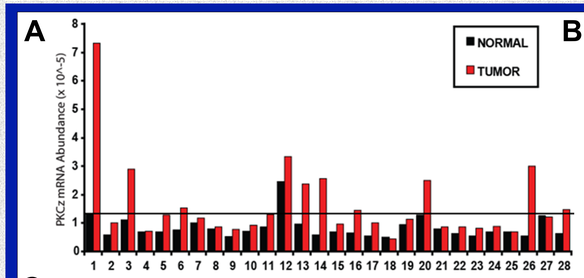
ABSTRACT:

Pancreatic cancer remains the fourth leading cause of cancer deaths in the United States due, in part, to its propensity for invasion and metastasis. Protein kinase C zeta (PKCζ) has been shown to regulate migration and invasion of both normal and malignant cell types. PKCζ has been shown to play a pro-carcinogenic role in various cancer types, including breast, colon, and glioblastoma; however, a role for PKCζ in the transformed growth of pancreatic cancer has not been demonstrated. We hypothesized that PKCζ may contribute to the lethality of pancreatic cancer by promoting cancer cell invasion and metastasis. Consistent with the hypothesis that PKCζ plays a role in the pancreatic cancer phenotype, PKCζ is overexpressed in a subset of human pancreatic tumors compared to associated non-tumor pancreas tissue. In the present study we use RNAi-mediated inhibition of PKCζ expression to investigate the role of PKCζ in the growth and invasion of pancreatic cancer cells. Inhibition of PKCζ reduces log phase growth and survival of human pancreatic cancer cell lines, with a corresponding reduction in colony formation in soft agar. Inhibition of PKCζ significantly reduces the size of tumors formed by human pancreatic cancer cells in a mouse orthotopic tumor model. Analysis of the isolated orthotopic tumors reveals a reduction in tumor cell proliferation in PKCζ RNAi tumors. In addition, PKCζ RNAi tumors exhibit elevated tumor necrosis compared to non-target RNAi (control) tumors. Furthermore, PKCζ RNAi tumors produce fewer metastases to distal organs, corresponding to the reduced migration and invasion of PKCζ RNAi cells *in vitro*. Signal transducer and activator of transcription 3 (STAT3), which is often constitutively activated in pancreatic tumors and pancreatic cancer cell lines, has been implicated in pancreatic cancer cell survival and metastasis. Indeed, we observe reduced cell survival, migration and invasion in pancreatic cancer cells in which STAT3 signaling is inhibited. Interestingly, inhibition of PKCζ significantly reduces constitutive STAT3 phosphorylation in pancreatic cancer cells *in vitro* and *in vivo*. Taken together these data strongly support a required role for PKCζ in pancreatic cancer cell survival, migration and invasion, and indicate that STAT3 may be a downstream effector of PKCζ in pancreatic cancer.



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Tumor PKCζ expression	Number	(percent)
Significantly reduced*	0	0
No change	20	(71)
Significantly elevated*	8	(29)

*= >2 standard deviations from average of PKCζ expression in non-tumor normal samples

Figure 1. PKCζ is elevated in a subset of human pancreatic tumors.

A) PKCζ expression is significantly increased in a subset of tumors compared with matched non-tumor tissue. quantitative PCR analysis of PKCζ mRNA expression was performed on 28 matched patient pancreatic tumor and non-tumor samples. PKCζ expression was normalized to 18S abundance. B) quantitation of A). C) PKCζ expression is significantly elevated in a subset of pancreatic tumors.

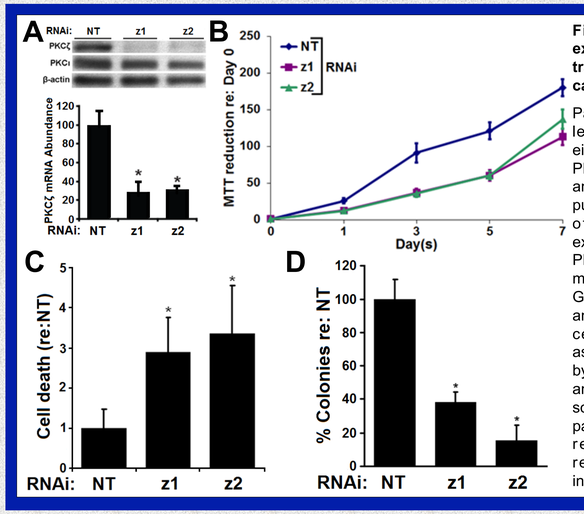
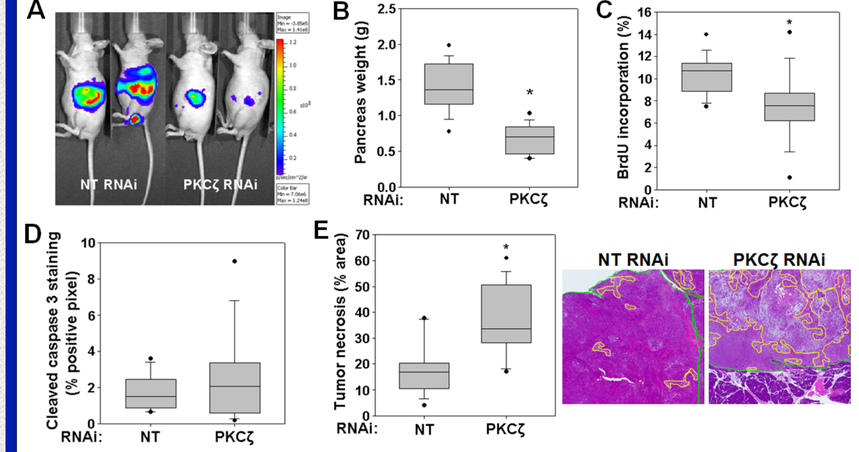


Figure 2. Inhibition of PKCζ expression reduces survival and transformed growth of pancreatic cancer cells.

Panc-1 cells were infected with lentiviral constructs expressing either control, non-targeting (NT) or PKCζ-targeting RNAi (z1 and z2) and stable populations selected with puromycin. A) Immunoblot analysis of PKCζ, PKCι and β-actin expression (top). qPCR analysis of PKCζ and PKCι mRNA expression. mRNA abundance is normalized to GAPDH (bottom). Panc-1 NT, z1 and z2 cells were assessed for B) cell growth (MTT colorimetric assay); C) basal cellular apoptosis by DNA break ELISA and D) anchorage-independent growth by soft agar colony formation. For each panel Bars=average of 6 or more replicates±SD and graph is representative of 3 or more independent experiments.



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% metastasis			
site	NT	PKCζ	*p*
liver	56	7	0.006
kidney	75	13	0.001
mesentery	63	0	0.0002
diaphragm	63	0	0.0002

Figure 3. Inhibition of PKCζ expression significantly reduces orthotopic pancreatic tumor size and tumor metastasis.

A) Representative bioluminescent imaging of mice with orthotopic Panc-1 NT and PKCζ RNAi pancreatic tumors. B) Weight of pancreas containing Panc-1 NT and PKCζ RNAi tumors. Quantitative analysis of tumor C) BrdU incorporation and D) cleaved caspase-3. E) Quantitative analysis of tumor necrosis (left). Representative H&E stained orthotopic Panc-1 NT and PKCζ RNAi pancreatic tumors with areas of necrosis identified (right, yellow outline). F) Mice with Panc-1 PKCζ RNAi orthotopic tumors developed significantly less metastases than mice with Panc-1 NT RNAi orthotopic tumors. n=15 NT RNAi tumors and 16 PKCζ RNAi tumors for all analyses in this figure.

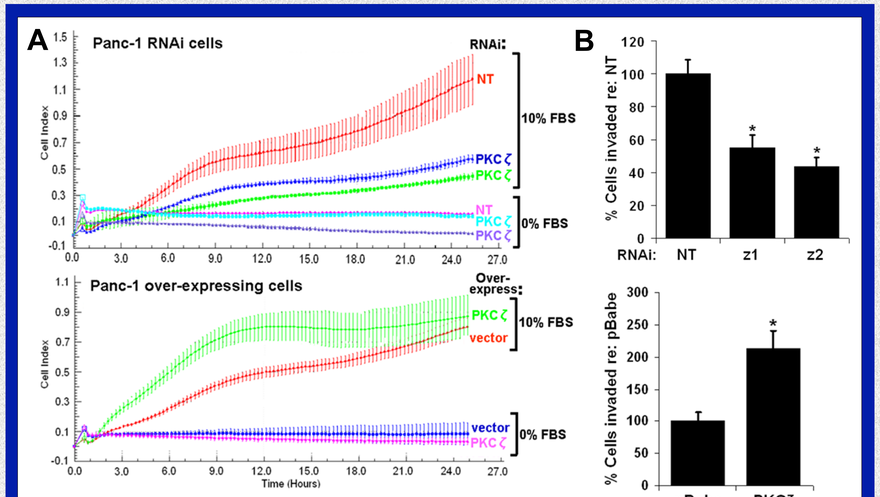

Figure 4. PKCζ expression regulates Panc-1 migration and invasion.

A) Cellular migration was assessed in Panc-1 NT and PKCζ RNAi cells (top) and Panc-1 vector-control and PKCζ overexpressing cells (bottom) in a real time cell migration assay. B) Cellular invasion through Matrigel-coated chambers was assessed in Panc-1 NT and PKCζ RNAi cells (top) and Panc-1 vector-control and PKCζ overexpressing cells (bottom).

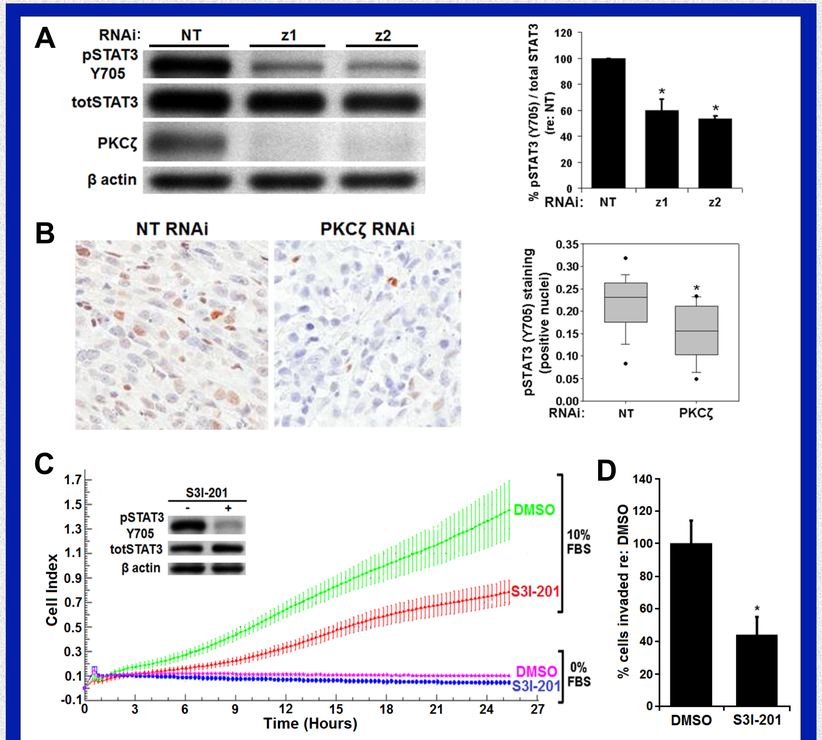

Figure 5. Inhibition of PKCζ expression significantly reduces STAT3 pY705 and STAT3 inhibition reduces Panc-1 migration and invasion.

A) Inhibition of PKCζ expression decreases constitutive pSTAT3 (Y705). Immunoblot analysis was performed on total cell lysates from Panc-1 NT and PKCζ RNAi cells (left). Quantitation of immunoblot (right). B) Representative IHC of pSTAT3 (Y705) in orthotopic Panc-1 NT and PKCζ RNAi pancreatic tumors (left). Quantitative analysis of pSTAT3 (Y705) IHC staining (right). C) Cellular migration was assessed in Panc-1 cells treated with STAT3 inhibitor (S3I-201, 100μM) or control (DMSO) in a Real time cell migration assay. D) Cellular invasion through Matrigel-coated chambers was assessed in Panc-1 cells treated with STAT3 inhibitor (S3I-201, 100μM) or control (DMSO).

CONCLUSIONS:

Our results demonstrate a requirement for PKCζ in the transformed growth in Panc-1 pancreatic cancer cells *in vitro* and *in vivo*. Our data demonstrate a role for PKCζ and STAT3 in Panc1invasion and migration. Our data suggest that PKCζ regulates STAT3 activation in pancreatic cancer cells. Taken together, our data implicate PKCζ as a candidate therapeutic target for the treatment of pancreatic cancer.

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