PROTEIN KINASE Cι IS REQUIRED FOR RAS TRANSFORMATION AND COLON CARCINOGENESIS IN VIVO

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ABSTRACT

PKCι has been implicated in Ras signaling; however, a role for PKCι in oncogenic Ras-mediated transformation has not been established. Here we show that PKCι is a critical downstream effector of oncogenic Ras in the colonic epithelium. Transgenic mice expressing constitutively active PKCι (caPKCι) in the colon are highly susceptible to carcinogen-induced colon carcinogenesis, whereas mice expressing kinase-deficient PKCι (kdPKCι) are resistant to both carcinogen- and oncogenic Ras-mediated carcinogenesis. Expression of kdPKCι in Ras-transformed rat intestinal epithelial (RIE/Ras) cells blocks oncogenic Ras-mediated activation of Rac1, cellular invasion and anchorage-independent growth. Constitutively active Rac1 (RacV12) restores invasiveness and anchorage-independent growth in RIE/Ras cells expressing kdPKCι. Our data demonstrate that PKCι is required for oncogenic Ras- and carcinogen-mediated colon carcinogenesis *in vivo* and define a pro-carcinogenic signaling axis consisting of Ras, PKCι and Rac1.

INTRODUCTION

The atypical PKC isozyme, PKCι, functions in the establishment of epithelial cell polarity and cell survival. PKCι plays a requisite role in Bcr-Abl-mediated malignancy in chronic myelogenous leukemia cells by conferring extreme resistance to chemotherapy-induced apoptosis. Ras is a proto-oncogene critical for cellular signaling pathways that regulate colonic epithelial cell proliferation, differentiation and apoptosis. Activating Ras mutations occur in ~30% of all human cancers, and in ~50% of human colon adenomas and carcinomas. Ras mutations have also been detected in early preneoplastic lesions, aberrant crypt foci (ACF), in both mice and humans, suggesting that activating Ras mutations play a role in ACF formation as well as in progression of colon adenomas to carcinoma. Whereas PKCι has also been implicated in Ras-mediated reorganization of the actin cytoskeleton and induction of cyclin D1 expression, nothing is known about its role in oncogenic Ras-mediated transformation. In the present study we investigate the role of PKCι in oncogenic Ras-mediated cellular transformation and carcinogenesis.

RESULTS

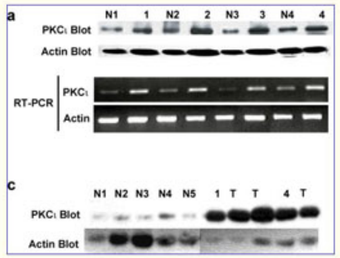

Figure 1: PKCι expression is elevated in mouse and human colon tumors. *a*) Immunoblot analysis for PKCι and actin. ***b***) RT-PCR analysis for mouse PKCι and actin mRNA. (T) AOM-induced mouse colon tumors and (N) uninvolved colonic epithelium from the same animals. ***c)*** Immunoblot analysis of lysates from colon tumor tissue (T) and matched, uninvolved (N) colonic epithelium from five patients with colon carcinoma.

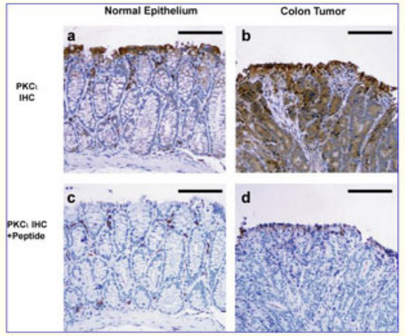

Figure 2: PKCι expression is elevated in AOM-induced colon tumors. Immunohistochemical analysis of sections from normal, uninvolved epithelium (*a* and ***c***) and an AOM-induced colon tumor (***b*** and ***d***) from the same animal in the absence (***a*** and ***b***) or presence (***c*** and ***d***) of 5-fold molar excess competing immunogenic peptide. Bars equal 50 µm.

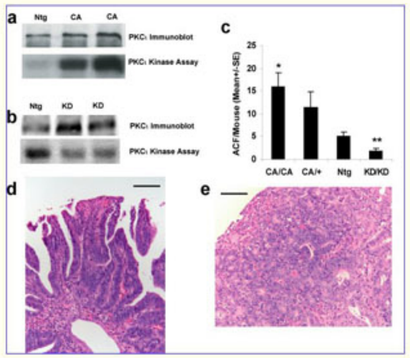

Figure 3: Transgenic caPKCι mice are highly susceptible to AOM-induced colon carcinogenesis. ***a)*** Immunoblot analysis and ***b)*** histone kinase assay of scraped colonic epithelium from non-transgenic (*Ntg*) and transgenic ***a)*** caPKCι (*CA*) or ***b)*** kdPKCι (*KD*) (***a*** and ***b***, ***upper panels***) and (*a* and ***b***, ***lower panels***). ***c)*** AOM-treated mice were scored for ACF. Results represent the average ACF/animal ± SEM (*n*=4-9; *p=0.05 versus Ntg; **p=0.02 versus Ntg). ***d)*** Tubular adenoma ***e)*** Carcinoma in situ. Bars equal 100 µm.

Table 1	% Tumor-bearing Mice (Tumor incidence)	Tumor Type		
		total tumors	Tubular adenoma	Intramucosal carcinoma
CA/CA transgenic	63.6% (7/11)	n=7	1	6
Non-transgenic	20% (2/10)	n=3	2	1

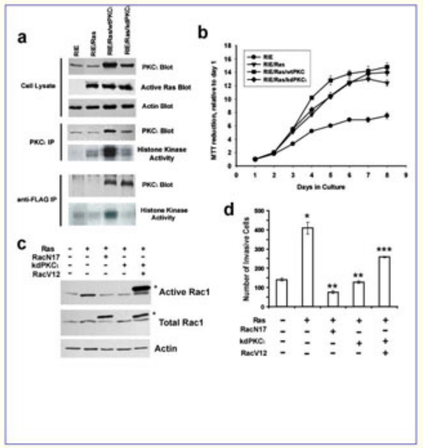

Figure 4: PKCι is required for oncogenic Ras-induced Rac1 activation and invasion *in vitro*. ***a)*** Characterization of RIE cell transfectants ***b)*** Anchorage-dependent growth of RIE cells and RIE cell transfectants. Data represent the mean ±SD from three independent determinations. ***c)*** Active (GTP-bound) Rac1 assay. The asterisk indicates the migration of Myc-tagged, virally-expressed Rac1 mutants. ***d)*** Cellular invasion assay. *p= 0.02 versus RIE + control vector; **p= or <0.02 versus RIE/Ras; ***p=0.005 versus RIE/Ras/kdPKCι.

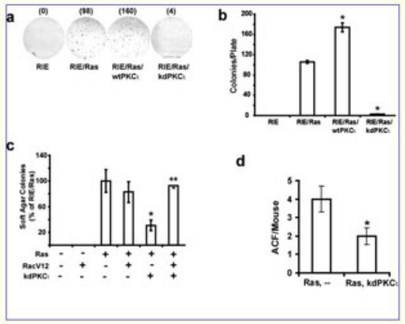

Figure 5: Expression of dnPKCι blocks Ras-mediated transformation of the intestinal epithelium *in vitro* and *in vivo*. ***a)-c)*** RIE cells transfectants were evaluated for growth in soft agar. ***a)*** Representative experimental results. ***b)*** Values represent the average of three independent experiments ± SEM. *p<0.002 versus RIE/Ras. ***c)*** Values represent the average of five determinations ± SEM. *p=0.008 versus RIE/Ras; **p=0.0001 versus RIE/Ras/kdPKCι. ***d)*** Twelve week old K-RasLA2 and K-RasLA2/kdPKCι mice were analyzed for ACF in the proximal colon. (*n*=5) *p=0.04.

CONCLUSIONS

1. **PKCι expression is induced in mouse and human colon tumors.**
2. **Oncogenic Ras activates PKCι and requires PKCι activity for Rac activation, cellular invasion and soft agar growth *in vitro*.**
3. **Expression of dominant-active Rac1 overcomes kinase-deficient PKCι inhibition of Ras-mediated cellular invasion and soft agar growth demonstrating that Rac1 is downstream of PKCι in these signaling pathways.**
4. **PKCι activity regulates susceptibility to carcinogen-induced preneoplastic lesion formation in the colon. Elevated colonic PKCι activity increases the number of carcinogen-induced colon tumors, and promotes tumor progression.**
5. **PKCι is critical for oncogenic K-ras-mediated colon carcinogenesis *in vivo*.**

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Nicole R. Murray and Lee Jamieson contributed equally to this work.