Genetic Ablation of PKC Epsilon Inhibits Prostate Cancer Development and Metastasis in Transgenic Mouse Model of Prostate Adenocarcinoma

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Abstract
Protein kinase C epsilon (PKCε), a novel PKC isoform, is overexpressed in prostate cancer (PCa) and correlates with disease aggressiveness. However, the functional contribution of PKCε to development or progression of PCa remained to be determined. Here we present the first in vivo genetic evidence that PKCε is essential for both the development and metastasis of PCa in the transgenic mouse model of prostate adenocarcinoma (TRAMP). Heterozygous or homozygous genetic deletions of PKCε in FVB/N TRAMP inhibited PCa development and metastasis as analyzed by positron emission tomography/computed tomography, tumor weight determinations, and histopathology. We also examined biomarkers associated with tumor progression in this model, including markers of survival, proliferation, angiogenesis, inflammation, and metastatic progression. To find clues about the genes regulated by PKCε and linked to the Stat3 signaling pathway, we carried out focused PCR arrays of JAK/STAT signaling in excised PCa tissues from PKCε wild-type and nullizygous TRAMP mice. Notably, PKCε loss was associated with significant downregulation of proliferative and metastatic genes C/EBPβ (CCAAT/enhancer binding protein β), CRP (C-reactive protein), CMK, EGFR (epidermal growth factor receptor), CD64, Jun B, and gp130. Taken together, our findings offer the first genetic evidence of the role of PKCε in PCa development and metastasis. PKCε may be potential target for prevention and/or treatment of PCa. Cancer Res; 71(6): 2318–27. ©2011 AACR.

Introduction
Prostate cancer (PCa) is the most common type of cancer in American men and ranks second to lung cancer in cancer-related deaths. The American Cancer Society has estimated that 217,730 new cases will be diagnosed and 32,050 deaths will occur in the United States alone in the year of 2010 (1). The lack of effective therapies for advanced PCa is related to a large extent to the poor understanding for the molecular mechanisms underlying the progression of the disease toward invasion and metastasis (2). Therefore, defining the novel molecular targets linked to PCa progression and metastasis will improve the planning strategies for the prevention and treatment of PCa.

PKC is a major intracellular receptor for the mouse skin tumor promoter 12-O-tetradecanoylphorbol-13-acetate. It represents a large family of phosphatidylinerse (PS)-dependent serine/threonine kinases (3–7). On the basis of structural similarities and cofactor dependence, 11 PKC isoforms have been classified into 3 subfamilies: the classic (cPKC), the novel (nPKC), and the atypical (aPKC). The cPKCs (α, β, γ) are dependent on PS, diacylglycerol (DAG), and Ca2+. The nPKCs (δ, ε, η, and ζ) retain responsiveness to DAG and PS but do not require Ca2+ for full activation. The aPKCs (δ, ε, ζ) require only PS for their activation (3–5). PKC epsilon (PKCε) is involved in the regulation of diverse cellular functions including gene expression, neoplastic transformation, cell adhesion, mitogenesis, and cell invasion (8, 9).

PKCε has been considered to be the hallmark of PCa development. Evidence suggests that overexpression of PKCε is sufficient to promote conversion of androgen-dependent LNCaP cells to androgen-independent (AI) variant, which rapidly initiates tumor growth in vivo in both intact and castrated athymic nude mice (10). A recent study has shown that overexpression of PKCε protected LNCaP cells against apoptotic stimuli via inducing phosphorylation of Bad at Ser112 residue (11). It has been shown that integrin signaling links PKCε to the PKB/AKT survival pathway in recurrent PCa cells (12). Proteomic analysis of PCa CWR22 cells xenografts show that association of PKCε with Bax may...
neutralize apoptotic signals propagated through the mitochondrial death signaling pathway (13). We and others have previously shown that PKCε level correlates with the aggressiveness of human PCa. Also, PKCε is overexpressed in PCa spontaneously developed in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice, an autochthonous transgenic model that mimics to the human disease (14). We have also shown that PKCε is a protein partner of transcription factor Stat3. PKCε associates with Stat3 and this association increases with the progression of the diseases in TRAMP mice and in human PCa. Taken together, all these findings suggest that PKCε is an oncogene and is involved in PCa development and aggressiveness and in the emergence of AI PCa (14). However, the role of PKCε in PCa development and progression in intact mouse model remains elusive. We present here for the first time that genetic loss of PKCε in TRAMP mice prevents development and metastasis of PCa, possibly via downregulation of proliferative and metastatic genes.

Materials and Methods

Antibodies

Monoclonal or polyclonal antibodies specific for Bcl-xL, β-actin, COX-2, gp130, GAPDH (glyceraldehyde 3-phosphate dehydrogenase), PKCα, PKCβ, PKCδ, PKCβII, PKCε, PKCy, PKCa, PKCζ, PKCs, PEK85, P3K110, PCNA (proliferating cell nuclear antigen), total Stat3α, total Stat5α, and VEGF were purchased from Santa Cruz Biotechnology. Antibodies specific to pAKT-ser473, pAKT-ser308, pAKTser473, and total AKT were purchased from Cell Signaling Technology.

Experimental animals

FVB-TRAMP mice were provided by Dr. Barbara Foster, Roswell Park Cancer Institute, Buffalo, NY. PKCε KO mice were generated and provided by Dr. Michael Leitges, Max-Plank-Institut Fur Immunologiologie, and Freiburg, Germany. To create the bigenic model, PKCε was crossbred with heterozygous (Het) null PKCε (WT/–, TRAMP-PKCεobtained and crossbred to generate the TRAMP-PKCε mice and in human PCa. Taken together, all these findings suggest that PKCε is an oncogene and is involved in PCa development and aggressiveness and in the emergence of AI PCa (14). However, the role of PKCε in PCa development and progression in intact mouse model remains elusive. We present here for the first time that genetic loss of PKCε in TRAMP mice prevents development and metastasis of PCa, possibly via downregulation of proliferative and metastatic genes.

Immuno blot analysis

Tissues from PCa and brains were excised and whole tissue lysates were prepared. In brief, part of the PCa and brain tissues of TG, Het, and KO mice were homogenized in lysis buffer (50 mmol/L HEPES, 150 mmol/L NaCl, 10% glycerol, 1% Triton X-100, 1.5 mmol/L MgCl2, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 1 mmol/L phenylmethanesulfonyl fluoride (PMSF), 200 μmol/L Na3VO4, 200 μmol/L NaF, and 1 mmol/L EGTA (final pH 7.5)). The homogenate was centrifuged at 14,000 × g for 30 minutes at 4°C. Supernatants were collected and stored at −80°C until further use. Protein was estimated using Bio-Rad protein assay kit as per the manufacturer’s protocol. Forty micrograms of tissue lysate were fractionated on 10% criterion precast SDS-polyacrylamide gel (Bio-Rad Laboratories). The protein was transferred to 0.45 μm Hydro-Blot polyvinylidene difluoride transfer membranes (Amersham Life Sciences). The membrane was then incubated with the indicated antibody followed by a horseradish peroxidase secondary antibody (Thermo Scientific), and the detection signal was developed with Amersham’s enhanced chemiluminescence reagent using FOTO/Analyst Luminary Work Station (Fotodyne Inc.). The Western blots were quantitated by densitometric analysis by using Totallab Nonlinear Dynamic Image analysis software (Nonlinear USA, Inc.).

Elevrofelectic mobility shift assay

Nuclear extracts were prepared from prostate tissues from TG, Het, and KO mice in a high-salt buffer [20 mmol/L HEPES (pH 7.9), 20 mmol/L NaF, 1 mmol/L Na2PO4, 1 mmol/L Na3VO4, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L DTT, 0.5 mmol/L PMSF, 420 mmol/L NaCl, 20% glycerol, 1 μg/mL leupeptin, and 1 μg/mL aprotinin]. The samples were then centrifuged and harvested. Protein was estimated by using protein assay kit (Bio-Rad) as per the manufacturer’s protocol. Stat3 DNA binding activity was determined in individual mouse nuclear lysates of each group. In brief, nuclear extracts (10 μg) were incubated in a final volume of 20 μL of 10 mmol/L HEPES (pH 7.9), 80 mmol/L NaCl, 10% glycerol, 1 mmol/L DTT, 1 mmol/L EDTA, and 100 μg/mL poly (deoxyinosinic-deoxycytidyllic acid) for 15 minutes. A P32-radioabeled double-stranded Stat3 consensus binding motif 5′-GATCCCTTCTGGAATTCTTAGATC-3′ (Santa Cruz Biotechnology) probe was then added and incubated for 20 minutes at room temperature. The protein–DNA complexes were resolved on a 4.5% nondenaturing polyacrylamide gel containing 2.5% glycerol in 0.1× Tris-glycine buffer, pH 8.3, and the autoradiograms were scanned and quantitated using Totallab Nonlinear Dynamic Image analysis software (Nonlinear USA, Inc.).
0.25 × Tris-borate EDTA at room temperature, and gels were dried and autoradiographed. Stat3 DNA binding activities were determined.

**Immunohistochemistry**

To determine the expression of Stat3, IL-6 receptor (IL-6R), and PCNA proteins in prostatic intraepithelial neoplasia (PIN) and moderately and poorly differentiated (PD) adenocarcinoma of each mouse of TG and KO, we conducted immunohistochemistry in paraffin-embedded sections (4-μm thickness). In brief, sections were deparaffinized by placing the slides at 60°C for 2 hours followed by 3 changes of xylene for 10 minutes each. Slides were placed in 0.3% methanol/hydrogen peroxide for 20 minutes for quenching endogenous peroxidase. Slides were rehydrated in one change of absolute, 95%, 75%, and 50% ethanol and distilled water. Antigen retrieval was carried out by incubating samples at 116°C for 15 seconds in the declocking chamber by using a Tris-borate solution (pH 9.5). After antigen retrieval, tissues slides were incubated with 2.5% normal horse serum (R.T.U. Vectorstain Universal Elite ABC Kit; Vector Laboratories) for 20 minutes to block nonspecific binding of the antibodies. Subsequently, the slides were incubated over night with a mixture of antibodies was decanted, blocking peptides of Stat3 and IL-6 antibodies were used of Stat3 (1:50), IL-6R (1:50), and PCNA (1:50) dilution in normal antibody diluents (Scy Tek # ABB-125) in a humidified chamber. Blocking peptides of Stat3 and IL-6 antibodies were used to determine the specific immunoreactivity of these antibodies. We further confirmed the specificity of immunostaining of PCNA, IL-6, and Stat3 by using IgG antibody (served as a negative control). The mixture of antibodies was decanted, and the slides were washed thrice in TBS (pH 7.4). The slides were incubated with appropriate secondary antibodies for 30 minutes at room temperature. Slides were rinsed with 0.05% ABC reagent (Vector kit) was applied for 30 minutes. Immunoreactive complexes were detected using 3,3′-diaminobenzidine substrate (Thermo Scientific) and counter stained by using hematoxylin (Fischer Scientific) for nuclear staining. Finally, slides were mounted with cover slip by using VECTABOND (Vector Laboratories). Stained slides were examined under a microscope and were photographed with an attached camera. A significant decrease in protein levels of PKCε was observed in the prostate of Het mice compared with TG mice (Fig. 1B). To determine whether deletion of PKCε has any compensatory effect in TRAMP mice, we carried out immunoblot analysis of PKC isoforms in prostate and brain tissue lysates of TG, Het, and KO mice. Results indicate no change in the expression of other PKC isoforms in brain tissue of Het and KO mice [Fig. IC (i and ii)], suggesting no compensatory effects in PKCε-deleted TRAMP mice.

**Deletion of PKCε in TRAMP mice inhibits PCa development and metastasis**

Accumulating evidence now indicates that PKCε is an oncogene which plays a vital role in the development of various types of human cancers including the prostate. Molecular genetic evidence of the role of PKCε in PCa development in an intact mouse model still remains obscure. In this study, we explored the possibility whether PKCε deletion in TRAMP mice inhibits the development and metastasis of PCa. A total of 21 mice (TG, n = 7; Het, n = 7; and KO, n = 7) were used in this study. In our first experiment, we carried out mini-PET/CT imaging, using a tumor selective radiopharmaceutical agent 124I-NM404 (16), of two 16 weeks old mice from each of TG and KO mice [Fig. 2A(i) and B(i)]. Results illustrated a lack of focal uptake of 124I-NM404 in KO mice [Fig 2B (i and ii)] compared with TG mice [Fig. 2A (i and ii)]. TG mice showed metastasis in proximal lymph node as evident by uptake of 124I-NM404 [Fig. 2A (i and ii)]. However, no metastasis was observed in KO mice, suggesting the role of PKCε in the development and metastasis of PCa. All of the remaining mice from each group were sacrificed at the same age (18 weeks). Their bloods were collected from retro-orbital plexus for serum isolation. PCa tissues were excised and parts of the tissues were used in preparation of whole tissue lysates, nuclear lysates, RNA isolation, and histology sectioning as

**Statistical analysis**

Student’s t test was carried out to determine the significance. P < 0.05 was considered as significant.

**Results**

**Generation and characterization of PKCε-deleted TRAMP mice**

As shown in Figure 1A, TG, Het, and KO mice were generated by cross-breeding 6 to 7 weeks old homozygous TRAMP with PKCε Het mice (Fig. 1A). Both TRAMP and PKCε KO mice were on FVB/N background. No PKCε expression was observed in either prostate or brain excised tissues of KO mice [Fig. 1B and C (i and ii)]. A significant decrease in protein levels of PKCε was observed in the prostate of Het mice compared with TG mice (Fig. 1B).
described in Material and Methods. Parts of the tissues excised from the kidneys, brains, livers, lungs, and lymph nodes were fixed in 10% buffered formalin and used for histopathology. Deletion of PKCε in TRAMP mice, or even one allele deletion, shows significant (P < 0.01) reduction in growth of PCa in all of the Het and KO mice (Fig. 2C and D). All TG mice developed 1 or 2 large-sized prostate tumors (Fig. 2C), whereas Het and KO mice had only a single small-sized tumor (Fig. 2C). One of the TG mice also showed grossly visible metastases in a local lymph node, both lungs, and the left kidney, which were confirmed by light microscopy (Fig. 2E and F). In addition, microscopic metastases were identified in another 3 TG mice. No metastasis was identified in any of the Het and KO mice (Fig. 2G and H). Hematoxylin and eosin (H&E)-stained tissue sections showed that all the grossly visible tumors were PD carcinomas characterized by solid sheets of large polymorphic cancer cells, with a high nucleus-to-cytoplasm ratio, frequent apoptosis, central necrosis, and neuroendocrine differentiation [Fig. 3D (i) and E (ii)]. Some of the Het and KO mice showed small foci of PD carcinoma at the microscopic level only. In addition, all the mice showed PIN characterized by epithelial cell proliferation with enlarged hyperchromatic nuclei and nuclear stratification in papillary and cribriform structures [Fig. 3D (i) and E (ii)].

**Deletion of PKCε in TRAMP mice inhibits Stat3 activation**

Aberrant activation of Stat3 has been linked to the progression of PCa metastasis (17–19). A study suggests that overexpression of Stat3 in normal prostate epithelial cells leads to conversion of malignant phenotype (20). To determine whether PKCε deletion in TRAMP mice inhibits Stat3 activation, we carried out immunoblot analysis in excised tissue PCa lysates of TG, Het, and KO mice. Results illustrated significant inhibition of both Ser727 and Tyr705 phosphorylation of Stat3 in both Het and KO mice (Fig. 3A). A significant decrease in DNA binding activity of Stat3 was observed in Het and KO mice [Fig. 3B (i and ii)]. Immunohistochemistry results show a significant decrease in intensity of nuclear staining of Stat3 in both Het and KO mice (Fig. 3F). A decrease in Stat3 staining in TG PCa tumor tissue was confirmed using blocking peptide of Stat3 (Fig. 3F).

**Deletion of PKCε inhibits serum IL-6 and IL-6R gp130 expression**

We have previously shown elevated levels of IL-6 in TRAMP mice compared with nontransgenic mice at the same age (14). To determine whether deletion of PKCε in TRAMP mice decreases serum IL-6 levels, we carried out specific ELISA
Deletion of PKCε inhibits markers of proliferation, antia apoptosis, and metastasis

Evidence from published studies, including our laboratory, suggests modulation of various apoptotic and proliferative biomarkers in PCa in both humans and TRAMP mice during the progression and metastasis (14, 21). To determine possible changes in biomarkers involved in apoptosis, proliferation, and metastasis of PCa in PKCε-deleted TRAMP mice, we conducted immunoblot analysis of selected biomarkers in PCa tissue lysates of TG, Het, and KO mice. We observed a decrease in the protein levels of Bcl-xL, COX-2, cyclin D1, and VEGF in Het and KO mice (Fig. 4A and B). We have previously shown overexpression of PI3K/AKT in PCa tissues of TRAMP mice compared with nontransgenic prostate tissues at the same age (14). We observed a decrease in the protein levels of regulatory subunit (p85β) of PI3K in Het and KO mice but no change was observed in the protein levels of catalytic isoform (p110β) of PI3K (Fig. 4B), pAKTSer473, or pAKTSer308 (Fig. 4C). We also carried out immunohistochemistry of PCNA in PCa tissues of TG and KO mice and observed a significant decrease in the nuclear levels of PCNA in PIN and PD PCa of KO mice [Fig. 4E (ii)–G].

Quantitative PCR array identifies decreased transcripts of genes implicated in PCa development and metastasis

To further define the role of PKCε in modulating the other genes associated with JAK/STAT3 signaling and involved in PCa development and metastasis, we carried out quantitative RT-PCR (qRT-PCR) array of genes associated with JAK/STAT signaling pathway on total RNA isolated from PCa tissues of TG and KO mice. This array contained 84 genes related to JAK/STAT3 signaling and involved in the progression and metastasis (14, 21). To determine possible changes in biomarkers involved in apoptosis, proliferation, and metastasis of PCa in both humans and TRAMP mice during the progression and metastasis (14, 21). To determine possible changes in biomarkers involved in apoptosis, proliferation, and metastasis of PCa in PKCε-deleted TRAMP mice, we conducted immunoblot analysis of selected biomarkers in PCa tissue lysates of TG, Het, and KO mice. We observed a decrease in the protein levels of Bcl-xL, COX-2, cyclin D1, and VEGF in Het and KO mice (Fig. 4A and B). We have previously shown overexpression of PI3K/AKT in PCa tissues of TRAMP mice compared with nontransgenic prostate tissues at the same age (14). We observed a decrease in the protein levels of regulatory subunit (p85β) of PI3K in Het and KO mice but no change was observed in the protein levels of catalytic isoform (p110β) of PI3K (Fig. 4B), pAKTSer473, or pAKTSer308 (Fig. 4C). We also carried out immunohistochemistry of PCNA in PCa tissues of TG and KO mice and observed a significant decrease in the nuclear levels of PCNA in PIN and PD PCa of KO mice [Fig. 4E (ii)–G].

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Discussion

In this study, we for the first time investigated PKCε link between the development and metastasis of PCa in transgenic
mouse model (TRAMP). We used a molecular genetic approach to generate bigenic TRAMP mice, which were also either wild-type, heterozygous null, or homozygous null for PKCe. Deletion of PKCe did not affect other isoforms of PKC in Het or KO mice, suggesting no compensatory effect of PKCe deletion in TRAMP mice. All of the PKCe-deleted TRAMP mice were healthy and we did not observe any gross pathologic changes in any organs of these animals.

TRAMP PCa closely mimics human PCa, as they both spontaneously develop progressive PCa. TRAMP PCa is invasive and capable of metastasis to distant sites, primarily to pelvic lymph nodes and to the lungs (22). Mouse genetic background affects the development of TRAMP PCa. TRAMP mice on FVB/N background more readily develop PIN compared with TRAMP mice on C57Black/B16 background (22). In our study, we observed that deletion of PKCe in TRAMP mice prevents tumor development, which was evident by a lack of focal uptake of $^{124}$I-NM404, a selective tumor agent [Fig. 2B (ii)]. This was followed by a significant (P < 0.01) decrease in prostate tumor weight in Het and KO animals (Fig. 2C and D).

Figure 3. Deletion of PKCe in TRAMP mice inhibits Stat3 activation. PCa tissues from TG, Het, and KO mice were excised and whole cell lysates were prepared and used for Western blot analysis as described in Materials and Methods. A, protein levels of pStat3Tyr705, pStat3Ser727, and total Stat3. Equal loading of protein was determined by stripping and reprobing the blots with β-actin antibody. Values in AN shown above the immunoblots represent quantitation of the bands normalized to β-actin as described in Materials and Methods. B, i, DNA binding activity of Stat3 in PCa tissues of TG, Het, and KO mice as determined by EMSA. Lane 1 is free probe. Specificity of Stat3 DNA binding was determined by mutant probe of Stat3 (lane 2). B, ii, quantitative analysis of EMSA of Stat3 DNA binding activity of TG, Het, and KO mice. C, bar graph represents quantification of Stat3 nuclear staining of TG and KO prostate tumor tissues. Student’s t test was carried out to analyze nuclear staining difference (P < 0.05). D, i through E, ii, representative photographs of (H&E) staining and immunohistochemistry of Stat3 in benign prostate (BP) epithelium, PIN, and PD adenocarcinoma of TG and KO mice. Arrows indicate the nuclear staining of Stat3. F, specificity of Stat3 antibody by using Stat3 blocking peptide.
Metastasis of PCa to distant organs is the main cause of morbidity and mortality in PCa patients. Importantly, we observed the metastasis of PCa to lungs, lymph nodes, and kidneys of the 70% TG animals, which was evident by the uptake of 124I-NM404 into the lymph nodes. Histopathologic examinations of lungs, lymph nodes, and kidneys of TG mice further confirm the metastasis of PCa. However, no metastasis was detected in any of the animals, even in the 2 mice from each Het and KO groups that had PCa. Taken together, these results strongly support that loss of PKCε inhibits both primary PCa and metastasis.

PCa is heterogeneous in nature, which involves activation of multiple signaling pathways. Constitutive activation of the transcription factor Stat3 has been shown to be a marker of poor prognosis in human PCa. Activation of Stat3 has been linked to the PCa development and metastasis through the induction of various genes responsible for tumor cell proliferation, cell survival, and carcinogenesis (17–19). In our previous studies, we have shown that PKCε activates and physically interacts with Stat3 in various types of human cancer cells including the prostate (23). In this study, loss of PKCε in TRAMP mice inhibited activation of Stat3, which...
was manifested by significant inhibition of both pStat3Ser727 and pStat3Tyr705 protein levels and decreased Stat3-DNA binding activity. Decrease in Stat3 nuclear staining was observed in PIN and PD adenocarcinoma of KO mice. These results are in accordance with our previously published reports, in which we have shown the interaction of PKCε with Stat3 during development of skin carcinoma in PKCε transgenic mice (24). These data further proves that PKCε may be an initiator for activation of Stat3 during PCa development. IL-6 is involved in activation of Stat3, which phosphorylates tyrosine residue of Stat3. Higher level of serum IL-6 has been associated with larger PCa burden, especially bone metastases (25, 26). In our study, KO mice showed a significant \( P < 0.05 \) decrease in serum IL-6 and its receptor gp130, which indicates that PKCε may have a direct or indirect role in potentiating the autocrine loop of IL-6 signaling in PCa. However, further detail investigation is warranted to understand the molecular mechanism of PKCε-mediated IL-6 signaling during the PCa development and metastasis. We further investigated various downstream target genes of Stat3 which have a role in the PCa inflammation, proliferation, angiogenesis, and metastasis (COX-2, cyclin D1, VEGF, Bcl-xL, IL-6R) which were significantly inhibited in KO mice. These data further provide evidence that PKCε is linked to activation of Stat3. PCNA is a nuclear

Table 1. List of genes that significantly modulated in KO mice compared with TG mice

<table>
<thead>
<tr>
<th>Name of the gene</th>
<th>Symbol</th>
<th>Fold decrease/ increase</th>
<th>Biological function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of genes downregulated in KO mice compared with TG mice</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP2</td>
<td>Cebpb</td>
<td>2.5</td>
<td>Cell proliferation and inflammation, upregulates metastatic genes</td>
</tr>
<tr>
<td>A0255847</td>
<td>Crp</td>
<td>4.34</td>
<td>Serum biomarker in various cancers, prognostic marker in castrated-resistant PCa</td>
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<td>CMK</td>
<td>CxCL9</td>
<td>2.94</td>
<td>T-cell trafficking</td>
</tr>
<tr>
<td>ERBB</td>
<td>EGFR</td>
<td>4.54</td>
<td>Member of Erbb2 family, cell proliferation, angiogenesis, invasion, metastasis, and inhibition of apoptosis, involved in angiogenesis and metastasis of PCa</td>
</tr>
<tr>
<td>Epor</td>
<td>Epor</td>
<td>3.57</td>
<td>Glycoprotein hormone, belongs to serine/threonine and tyrosine family regulators of red blood cells in mammals, overexpressed in PCa tissues</td>
</tr>
<tr>
<td>CD64</td>
<td>Fcgr1</td>
<td>2.56</td>
<td>Stimulates cell proliferation of macrophages, develops resistance to chemotherapeutic drugs, and radiation therapy</td>
</tr>
<tr>
<td>UCRP</td>
<td>Isg15</td>
<td>3.0</td>
<td>Interferon-regulated protein involved in cell growth, cell cycle</td>
</tr>
<tr>
<td>GHR</td>
<td>Ghr</td>
<td>2.77</td>
<td>Involved in tumor cell growth of PCa</td>
</tr>
<tr>
<td>mIL-10R</td>
<td>Il10ra</td>
<td>3.12</td>
<td>Cell proliferation, differentiation, and activates transcription factors Stat1 and Stat3</td>
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<tr>
<td>IL-10R2</td>
<td>Il10rb</td>
<td>3.70</td>
<td>Activates JAK proteins</td>
</tr>
<tr>
<td>CD132</td>
<td>Il2rg</td>
<td>2.70</td>
<td>Activates JAK proteins</td>
</tr>
<tr>
<td>CD124</td>
<td>Il4ra</td>
<td>2.22</td>
<td>Regulates inflammatory, cell-mediated immune response</td>
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<tr>
<td>gp130</td>
<td>Il6st</td>
<td>2.5</td>
<td>Member of IL-6/Stat3 signaling</td>
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<tr>
<td>Jun B</td>
<td>Jun B</td>
<td>2.5</td>
<td>Member of Fos/Jun family, activates AP1 transcription factor</td>
</tr>
<tr>
<td>L3</td>
<td>Oas1a</td>
<td>2.22</td>
<td>Involved in immune response</td>
</tr>
<tr>
<td>CD140a</td>
<td>Pdgfra</td>
<td>2.70</td>
<td>Receptor that bind and activates JAK proteins, involved in angiogenesis</td>
</tr>
<tr>
<td>Cd45</td>
<td>Ptprc</td>
<td>2.77</td>
<td>Receptor that bind and activates JAK proteins</td>
</tr>
<tr>
<td>SOCS-1</td>
<td>sos1c</td>
<td>2.63</td>
<td>Involved in cell growth</td>
</tr>
<tr>
<td>Aprf</td>
<td>Stat3</td>
<td>2.85</td>
<td>Involved in cancer cell survival, inhibition of apoptosis, invasion, and metastasis</td>
</tr>
<tr>
<td><strong>No. of genes upregulated in KO mice compared with TG mice</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>Il4</td>
<td>2.26</td>
<td>Involved in apoptosis</td>
</tr>
<tr>
<td>AP-1</td>
<td>Jun</td>
<td>2.83</td>
<td>Cell differentiation</td>
</tr>
<tr>
<td>Pr-1</td>
<td>Prfr</td>
<td>3.58</td>
<td>Lymphocyte activation</td>
</tr>
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NOTE: Deletion of PKCε in TRAMP mice modulates IL-6/Stat3 signaling–associated genes involved in PCa progression and metastasis. Focused qPCR array for JAK/STAT signaling pathway was carried out in TG and KO PCa as described in Materials and Methods. Table represents the fold increase or decrease in mRNA expression of JAK/STAT signaling–associated genes in KO mice compared with TG mice.
protein, which has been known as a cell proliferative marker, and a decrease in the expression of PCNA reflects cell-cycle arrest in G$_1$/S phase (27). We observed a significant decrease in the expression of PCNA in KO mice. These data correspond to the previous study, which has shown that knockdown of PKC$_\varepsilon$ in Pca LNCaP cells arrested the cell cycle in G$_1$/S phase (10). We also conducted focused qRT-PCR arrays of genes involved in the JAK/STAT signaling pathway in TG and KO Pca tissues to determine the role of PKC$_\varepsilon$ in JAK/STAT signaling. A significant decrease in mRNA expression of CEBP$_\alpha$, CRP, EGFR, gpl30, Jan B, and Stat3 was observed in KO mice compared with TG mice (Table 1). These results further provide clues about the role of PKC$_\varepsilon$ in activation of Stat3 and other signaling molecules linked to the Pca development and metastasis. However, a detailed investigation is warranted to find out the cross-talk of PKC$_\varepsilon$ with JAK/STAT signaling pathway–associated molecules.

In summary, deletion of PKC$_\varepsilon$ in FVB/N TRAMP mice inhibits Pca development and metastasis. We have previously reported that Stat3 is a protein partner of PKC$_\varepsilon$ (14, 23). PKC$_\varepsilon$ interacts with Stat3 and phosphorylates Stat3Ser727, essential for Stat3 activation and nuclear localization (14). In consistent with previous findings, deletion of PKC$_\varepsilon$ in FVB/N TRAMP mice downregulated prostatic Stat3 activation and Stat3-regulated gene expressions. We conclude that PKC$_\varepsilon$ and its downstream protein partner Stat3 constitute essential components of the signal transduction pathways involved in Pca development and metastasis. Therefore, we suggest that targeting PKC$_\varepsilon$ is a novel approach for prevention or treatment of Pcas.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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