Protein Kinase D1 Inhibits Cell Proliferation through Matrix Metalloproteinase-2 and Matrix Metalloproteinase-9 Secretion in Prostate Cancer

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Abstract

We and others previously showed that protein kinase D1 (PKD1) is downregulated in several cancers including prostate; interacts with E-cadherin, a major cell adhesion epithelial protein; and causes increased cell aggregation and decreased motility of prostate cancer cells. In this study, we show that PKD1 complexes with α3-integrin, resulting in activation of mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase–ERK pathway, which causes increased production of matrix metalloproteinase (MMP)-2 and MMP-9, that is associated with shedding of soluble 80 kDa E-cadherin extracellular domain. Interestingly, decreased cell proliferation following PKD1 transfection was rescued by MMP-2 and MMP-9 inhibitors and augmented by recombinant MMP-2 (rMMP-2) and rMMP-9 proteins, suggesting an antiproliferative role for MMPs in prostate cancer. Translational studies by in silico analysis of publicly available DNA microarray data sets show a significant direct correlation between PKD1 and MMP-2 expression in human prostate tissues. The study shows a novel mechanism for antiproliferative effects of PKD1, a protein of emerging translational interest in several human cancers, through increased production of MMP-2 and MMP-9 in cancer cells. Cancer Res; 70(5): 2095–104. ©2010 AACR.

Introduction

The basement membrane and transmembrane proteins contain signals for cell survival and growth, but loss of these signal results in cell death or suppression of proliferation in both normal and cancerous cells (1, 2). Matrix metalloproteinases (MMP) belong to a family of proteinases, which play a role in the degradation of basement membrane or transmembrane proteins, which can lead to loss of cell survival and growth signals (3). MMPs are neutral proteinases secreted from various cells as inactive zymogens and activated by Zn or Ca ion-dependent proteolytic cleavage that catalyze the destruction of ECM and transmembrane proteins (4, 5). There are 24 known isoforms of MMPs to date (6), and most have been shown to contribute to cancer progression and metastasis (7). However, MMPs might also negatively regulate cancer cell survival; MMP-3 and MMP-7 have been shown to have proapoptotic function in epithelial cells (8–10). Other MMPs, such as MMP-9, can suppress the proliferation of T lymphocytes through disruption of interleukin-2Rα signaling (11), MMP-8–deficient mice exhibit an increased tumor susceptibility compared with wild-type mice (12), and active MMP-2 promotes apoptosis of hepatic stellate cells by cleavage of N-cadherin (13). Moreover, mice deficient in MMP-2, MMP-3, or MMP-9 have lower level of apoptosis induced by tumor necrosis factor-α (TNF-α; ref. 14).

Several studies have shown an association between increased MMPs production and malignant progression of prostate cancer, with increased MMP-2 and MMP-9 associated with higher grade prostate cancer (15). However, whether MMP-2 and MMP-9 play a proapoptotic or prosurvival function in prostate cancer cells is unclear. In addition, there is paucity of data with regard to regulators of MMP secretion and activity in prostate cancer. In this study, we describe protein kinase D1 (PKD1) as a novel regulator of increased secretion of MMP-2 and MMP-9 in prostate cancer cells, which causes suppression of prostate cancer cell proliferation and colony formation, suggesting a tumor suppressive role for MMP-2 and MMP-9 in prostate cancer.

PKD1 was originally cloned and termed PKCμ and later reclassified as charter member of new proteins family called PKD, which comprises of a family of two other structurally closely related isoforms, PKD2 and PKD3. PKD1 is associated with plethora of cellular functions, including cell growth, cell survival, invadopodia formation, Golgi organization, and trafficking (16). Additionally, PKD1 interacts with integrin, a heterodimeric transmembrane receptor that mediates interactions between cells and ECM and transmit signals across the plasma membrane that influence a range of biological processes, including cell proliferation, migration, and...
and invasion in prostate cancer cells and functions epistatically with E-cadherin, a major transmembrane cell-cell adhesion protein and a classic marker of epithelial cells (22). Our laboratory has also shown that PKD1 inhibits cell proliferation, motility, and invasion in prostate cancer cells and functions epistatically with E-cadherin, which is mediated by β-catenin, a major binding partner in cadherin-catenin complex of proteins (23).

E-cadherin, a member of classic cadherins, plays an important role in tissue morphogenesis, wound healing, and maintenance of tissue integrity (24, 25). The extracellular domain of E-cadherin interacts homotypically with cadherins on the surface of neighboring cells to form calcium-dependent adherence junctions. Cadherin-mediated adhesion must be dynamic to accommodate epithelial growth and remodeling during development and to facilitate wound healing and turnover of epithelia in mature tissues, which can be accomplished by E-cadherin shedding (25). The shedding occurs following cleavage of extracellular domain of E-cadherin by proteases, including ADAM, γ-secretase, and MMPs (26–28). However, the exact isoforms of MMPs involved in E-cadherin shedding is yet to be defined. Proteolytic cleavage of E-cadherin has been suggested to be a cause of rapid changes in cell adhesion, signaling, anoikis, and apoptosis (26–28).

In this study, we show that PKD1 is a novel regulator in MMP-2 and MMP-9 production as well as shedding of E-cadherin in prostate cancer cells. PKD1 interacts with β3-integrin to regulate extracellular signal-regulated kinase (Erk) phosphorylation resulting in increased secretion of MMP-2 and MMP-9, suppresses prostate cancer cell proliferation as well as colony formation, and increases E-cadherin shedding. In silico analysis of publicly available human tissue gene expression data sets confirmed a significant direct correlation between PKD1 and MMP-2 expression.

**Materials and Methods**

**Cell culture, plasmid preparation, and cell transfection.** Human prostate cancer cell lines PC3 and DU145 (American Type Culture Collection) were grown in RPMI 1640 with 10% fetal bovine serum (FBS). pEGFP and pEGFP-PKD1 plasmids were prepared as previously described (29). PC3 and DU145 cells were transfected with pEGFP and pEGFP-PKD1 plasmids by Lipofectamine 2000 (Invitrogen), and clones were selected by Geneticin (Invitrogen). The knockdown of PKD1 by siRNA has been described previously (29, 30). Erk was knocked down by transfection of SignalSilence (Invitrogen). The knockdown of PKD1 by shRNA has been described previously (29, 30). Erk was knocked down by transfection of SignalSilence p44/42 mitogen-activated protein kinase (MAPK) siRNA (cell Signaling), and SignalSilence Control siRNA (Cell Signaling) was used as control siRNA.

**Reagents and chemicals.** Erk, p-Erk (Thr202/Tyr204), p-Mek1/2 (Ser217/221), and β-integrin antibodies were purchased from Cell Signaling; green fluorescent protein (GFP), PKD1 (clone C-20), IgG, and E-cadherin (H-108) antibodies from Santa Cruz Biotechnologies and Type-A Gelatin from Sigma-Aldrich, Inc. Horseradish peroxidase and fluorescence conjugated anti-IgGs were purchased from Jackson ImmunoResearch Laboratories, anti-MMP-2 and anti-MMP-9 were from Chemicon International, recombinant proteins of MMP-2 and MMP-9 as well as inhibitor of MMP-2 and MMP-9 were from Calbiochem, and MTS reagents were from Promega.

**Assay for MMP-2 and MMP-9 activity by zymography.** The activities of MMP-2 and MMP-9 in the conditioned media were assayed by zymography as described previously (31). Briefly, conditioned media were subjected to gel electrophoresis containing 0.3% gelatin. Then gels were washed and incubated for 18 h at 37°C in the reaction buffer [50 mmol/L Tris-HCl (pH 7.4), 10 mmol/L CaCl2], followed by staining and destaining.

**Electrophoresis, immunoblotting, and immunoprecipitation.** Electrophoresis, immunoblotting, and immunoprecipitation were performed as described elsewhere (29, 32). Briefly, proteins were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membrane. The membrane was blocked and incubated with respective antibody, followed by incubation with secondary antibody. Proteins were visualized by enhanced chemiluminescence (Amersham). For immunoprecipitation of specific proteins, cells lysates were incubated with respective antibody, followed by incubation with protein A-Sepharose beads (Thermo Scientific). Beads were subsequently washed and extracted the protein with 2× sample buffer.

**Cell proliferation assay (MTS).** Cells were seeded at a density of 1,000 cells per well in 96-well plates for 24 h, and MTS assay was performed according to manufacturer’s recommendation (Promega).

**[3H]Thymidine incorporation assay.** GFP or PKD1 stably expressed DU145 cells were plated (5 × 103 per well) in 24-well plates with complete medium; serum starved overnight; treated with DMSO, 5 μmol/L MMP-2 inhibitor, or recombinant MMP-2 (rMMP-2) and MMP-9 (300 ng/mL) proteins; labeled with [3H]thymidine (0.25 μCi/well); incubated for 48 h; washed sequentially with ice-cold PBS, 5% ice-cold trichloroacetic acid with 5 min incubation on ice, ice-cold PBS; and solubilized in a solution of 200 μL 0.5% SDS/0.5 N NaOH. Then 150 μL of cell solution was mixed with 3 mL scintillation fluid and counted by liquid scintillation counter.

**RNA isolation and semiquantitative reverse transcription–PCR.** Total RNA was isolated from respective cells using TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. RNA (1 μg) from each sample was reverse-transcribed using Superscript II (Invitrogen). Relative expression level of proliferating cell nuclear antigen (PCNA) was determined by semiquantitative PCR. The PCNA primers were 5′-GGCTGTAACCTCCAGCATT-3′ (forward) and 5′-TGTCCCCATATCCGCAATTT-3′ (reverse). Specific primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5′-TACCACTTCTCAGGAGC-3′ (forward) and 5′-GGAATTAGATTGTCTGGAGACC-3′ (reverse). Platinum-Taq DNA polymerase (Invitrogen) was used in all amplification reactions to minimize nonspecific product amplification. The number of amplification cycles was 24 for PCNA and GAPDH.

**Condensation of conditioned media.** Condensation of conditioned media was performed as described previously.
Briefly, cells were seeded on 6-cm dish at a concentration of $1 \times 10^6$ cells per dish. After 24 h of incubation, medium was changed to serum-free RPMI and incubated for additional 24 h. Then the conditioned media were collected, and secreted proteins were ethanol precipitated and dissolved in 2× sample buffers for Western blotting.

**Immunofluorescence.** Cells were cultured on coverslips until subconfluence and processed for immunofluorescence study. The coverslips were incubated with primary antibodies in 10% FBS in PBS. After washing with PBS, coverslips were incubated with fluorescein isothiocyanate and conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) for 1 h. The cells were washed in PBS and mounted on slides with Slow Fade Antifade reagent (with 4',6-diamidino-2-phenylindole). Photographs were taken at 400× magnification using Olympus fluorescent microscope followed by analysis with imaging software (Photoshop).

**Soft agar colony assay.** Cells ($5 \times 10^4$) were mixed with 0.36% agar in RPMI supplemented with 10% FBS and overlaid onto a 0.72% agar layer in six-well plates. After 3 wk of incubation, colonies in randomly selected fields (400× magnification) were counted.

**Data mining and bioinformatics analysis.** Three data sets (GSE3325, GSE6919, and GSE6099) were downloaded\(^4\) and analyzed using the statistical language R (34). All data were log transformed using the robust multichip average algorithm. Normalized expression values were used to plot the data. Scatter plot was overlaid on linear regression lines.

**Results**

**PKD1 increases production of MMP-2 and MMP-9 in DU145 and PC3 cells.** To explore the role of PKD1 in production of MMP-2 and MMP-9, we first examined the condition media from DU145 and PC3 cells that were stably expressed GFP or PKD1-GFP (Fig. 1A) for MMP-2 and MMP-9 secretion.

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by zymography. As shown in Fig. 1B, PKD1-GFP–transfected cells show increased secretion and activation of MMP-2 or MMP-9 compared with GFP-transfected and parental cells. These results strongly suggest that PKD1 is a positive modulator for secretion and activation of MMP-2 or MMP-9 in DU145 and PC3 prostate cancer cells. To clarify whether the PKD1-induced secretion of MMP-2 and MMP-9 in prostate cancer cells is associated with intracellular expression of MMP-2 and MMP-9, we immunoblotted cell lysates with MMP-2 and MMP-9 antibodies. As shown in Fig. 1C, PKD1 increased expression of MMP-2 and MMP-9 in DU145 and PC3 cells compared with GFP-transfected and parent cells. Our results suggest that PKD1 upregulated the expression and secretion of MMP-2 and MMP-9. As a corollary, DU145 cells were transfected with shLuc as control or shPKD1 to knockdown expression of PKD1 showed that secretion of MMP-2 and MMP-9 were suppressed in the PKD1 knockdown DU145 cells by 68% and 55%, respectively, compared with shLuc-transfected control cells (Fig. 1D).

**Phosphorylation of MAPK/ERK kinase and Erk is required for PKD1-induced secretion of MMP-2 and MMP-9.** Previous reports suggest that MMP-2 or MMP-9 secretion is regulated by Ras-dependent Erk pathway (31, 35). To find out the downstream effectors of PKD1 signaling on secretion of MMP-2 and MMP-9, we studied the activation of MAPK/ERK kinase (Mek) and Erk in PKD1-GFP, GFP-expressing, and parental PC3 and DU145 cells by immunoblotting with phosphorylated-specific antibodies for Mek and Erk proteins. The activated form of Mek and Erk proteins was increased in PKD1-GFP–expressing cells compared with parental and GFP-expressing PC3 and DU145 cells (Fig. 2A). Alternatively, knockdown of PKD1 by shRNA in DU145 cells decreased the phosphorylation of Erk (Fig. 2B), suggesting that PKD1 increases activation of Erk and Mek proteins.

Figure 2. Mek-Erk phosphorylation is necessary for PKD1-induced MMP-2 and MMP-9 secretion in PC cells. A, nontransfected (Cont.), GFP-transfected, and PKD1-GFP–transfected PC3 and DU145 cell lysates were immunoblotted with indicated antibodies. B, DU145 cells were transfected with shLuc and shPKD1 constructs. After 24 h, the cells were lysed and immunoblotted with PKD1, β-actin, p-Erk, or Erk antibodies. The relative densitometric data of band intensity for p-Erk is shown at the bottom of each panel. C, PKD1-transfected stable PC3 cells were incubated with DMSO (Cont.) or U0126 (5 μmol/L) for 1 h, and cell lysates were immunoblotted with p-Erk, Erk, PKD1, or β-actin antibodies. Conditioned media were collected from each condition and subjected to gelatin zymography. Mean value of densitometric analysis of three independent experiments was shown in the chart (bottom). Bar, SD of mean. *, P < 0.05. D, PC3-PKD1 cells were transfected with control siRNA or siErk. After 24 h of transfection, cells were lysed and immunoblotted with Erk and β-actin antibodies. Conditioned media were collected from each condition and subjected to gelatin zymography (bottom of each panel). Relative densitometric data of band intensity were shown at the bottom of each panel.
To confirm the role of Erk in PKD1-induced secretion of MMP-2 and MMP-9, we used specific MEK inhibitor, as well as siErk, to examine the effect on MMP-2 and MMP-9 secretion in PC3-PKD1 cells. Incubation of PC3-PKD1 cells with U0126, a specific inhibitor of MEK, completely blocked the PKD1-induced activation of Erk without altering PKD1 expression (Fig. 2C). The secretion of MMP-2 and MMP-9 was also suppressed by 31% and 70%, respectively, with U0126 compared with control cells (Fig. 2C). Similarly, knockdown of Erk suppressed MMP-2 and MMP-9 secretion remarkably (Fig. 2D). These biochemical and genomic experimental data clearly show that PKD1-induced Erk activity influences MMP-2 and MMP-9 secretion.

**PKD1–β3-integrin complex regulates the secretion of MMP-2 and MMP-9.** Several groups have reported that PKD1 complexes with β3-integrin and regulates various signaling pathways (19, 20), including the Erk and Akt activation (36, 37). We explored the presence of PKD1–β3-integrin protein complex in PC3 cells by immunoprecipitation and found that PKD1 and β3-integrin antibodies could immunoprecipitate β3-integrin and PKD1, respectively (Fig. 3A), in prostate cancer cells. Moreover, PKD1 and β3-integrin colocalized in the presence of β3-integrin mimic peptide RGE, which was abolished following treatment with RGD peptide, a highly specific β3-integrin inhibitor (Fig. 3B), suggesting that PKD1 and β3-integrin exist in the same protein complex in vivo.

We also observed that the inhibitory RGD peptide suppressed the phosphorylation of Erk in PC3-PKD1 cells in a dose-dependent manner without altering PKD1 expression (Fig. 3C), whereas treatment with control RGE peptide did not show any such effect. The secretion of MMP-2 and MMP-9 was also suppressed by the RGD peptide, but not by RGE peptide (Fig. 4D). Quantitative analysis showed that...
1 and 5 μmol/L RGD peptide suppressed MMP-2 and MMP-9 by 37% and 35% and by 57% and 59%, respectively, compared with control RGE peptide, suggesting a dose-dependent response. Collectively, our results suggest that PKD1–β3-integrin complex is a signaling molecular complex that induces phosphorylation of Erk, resulting in increased secretion of MMP-2 and MMP-9 in prostate cancer cells.

**PKD1-induced secretion of MMP-2 and MMP-9 increases E-cadherin shedding and suppresses cell proliferation.** MMPs are known to cause E-cadherin shedding by proteolysis of extracellular domain (38). We explored whether increased secretion of MMP-2 and MMP-9 by PKD1 resulted in the shedding of soluble E-cadherin (sE-cad) in conditioned media, which can be used as a read out of MMP activity. Overexpression of PKD1 enhanced production of sE-cad in conditioned media of DU145 cells compared with GFP-transfected and parental cells (Fig. 4A). Incubation of PKD1-DU145 cells with DMSO, MMP-2, or MMP-9 inhibitors suppressed sE-cad in conditioned media (Fig. 4B). PKD1 suppressed proliferation of DU145 cells, which was interestingly rescued by treatment with MMP-2 and MMP-9 inhibitors (Fig. 4C and D). In contrast, MMP inhibitor has no effect in GFP-DU145 cells (Fig. 4C and D). These results were corroborated by reverse transcription–PCR (RT-PCR) of PCNA, a cell proliferation marker under same conditions (Fig. 4C, bottom). The results suggest that MMP-2 and MMP-9 could mediate PKD1-induced suppression of cell proliferation in DU145 cells.

**rMMP-2 and rMMP-9 proteins suppress anchorage-independent and anchorage-dependent growth of PKD1-overexpressing prostate cancer cells.** To further confirm the inhibitory role of MMP-2 and MMP-9 on cell proliferation, GFP-overexpressed and PKD1-overexpressed DU145 cells were incubated with rMMP-2 and rMMP-9. The results showed that rMMP-2 and rMMP-9 suppressed the proliferation of PKD1-overexpressing cells only (Fig. 5A and B) and also inhibited anchorage-independent growth on soft agar by significant reduction in number of colonies in PKD1-DU145 cells compared with controls (Fig. 5C).

**Expression of PKD1 directly correlates with MMP-2 expression in human prostate tissue.** We interrogated publicly available DNA microarray expression data sets derived from control, GFP-overexpressing, and PKD1-GFP–overexpressing DU145 cells and immunoblotted with E-cadherin antibody (first panel). Lysates from same cells were immunoblotted with E-cadherin and β-actin antibodies. B, PKD1-overexpressing DU145 cells were incubated with DMSO (Cont.), 5 μmol/L MMP-2 inhibitor, or 200 nmol/L MMP-9 inhibitor for 24 h, and conditioned media were immunoblotted with E-cadherin antibody. C and D, MTS and [3H]thymidine incorporation assay were performed with GFP-overexpressing and PKD1-GFP–overexpressing DU145 cells. The cells were incubated with DMSO (Cont.), inhibitor of MMP-2 or MMP-9 for 48 h. Cell proliferation was measured by MTS and [3H]thymidine incorporation assay and expressed as a percentage of control. Data are the means of three independent experiments with triplicate samples. Bar, SD of mean. *, P < 0.05. Results from RT-PCR of PCNA and GAPDH from the cells treated with MMP-2 and MMP-9 inhibitors are also shown (C, bottom).

![Image](https://cancerres.aacrjournals.org/content/70/5/2100/F4.large.jpg)
from human prostate benign and cancerous tissues using search terms PRKD1 (PKD1), MMP-2, and MMP-9 in National Center for Biotechnology Information’s Gene Expression Omnibus. We focused initially on data sets studying benign, primary, and metastatic prostate cancer. Three data sets (GSE3325, GSE6919, and GSE6099) were identified, and the gene expression data were compared. As we previously reported (21), expression of PRKD1 was reduced in metastatic samples in all data set analyses, but not in benign and primary tumor samples (Fig. 6; data from two of three data sets shown). Similarly, MMP-2 gene expression was also downregulated in prostate metastatic samples only. As shown in Fig. 6B, we observed a significant positive correlation between PRKD1 and MMP-2 across all samples with \( r = 0.78 \) and \( P < 0.05 \). Surprisingly, the expression of MMP-9 did not significantly correlate with PKD1 gene expression \( (r = -0.18, P > 0.05) \).

The heat map plots the expression values (GSE6919) for the genes under consideration in each of the microarray chips used in this study. It confirms the behavior of PRKD1 stated earlier, in that the individual heat spots (expression values) tend toward the blue color (lower values) when expressed in normal, tumor, or metastatic prostate tissues. Similarly, the expression signals for MMP-2 tend to become smaller as the prostate tissue transforms to the metastatic state. In the case of MMP-9, only a weak trend in the opposite direction can be observed (Fig. 6C). There are more red spots as the tissue moves to the metastatic state.

**Discussion**

The association between MMPs and cancer can be traced back to 1970s, and in fact, several MMP inhibitors have been evaluated clinically with very limited success (39). Arguably, several reasons are cited for failure of MMP inhibitors in clinical setting, including incomplete understanding of various MMP isoforms and their functions. Whereas initially MMPs were thought to play a major role in cancer progression,
there is emerging data that show several isoforms of MMPs also show antitumor properties (9, 10). MMPs can perform antitumor functions by stimulating apoptosis, regulating inflammation, angiogenesis inhibition, metastasis suppression, and altered hormonal signaling (40). In this study we show that MMP-2 and MMP-9 inhibit proliferation of prostate cancer cells. MMP-9, MMP-3, MMP-11, and MMP-19 are examples of dual proteases that show protumor and antitumor roles, which are context dependent (39). We have identified MMP-2 as another MMP that shows antitumor properties in prostate cancer.

MMPs are well known to cleave cell surface proteins, including adhesion molecules, apoptotic protein, cell surface receptors, cytokines, growth factors, proteases, intercellular junctional, and structural proteins with important consequences on cellular phenotypic behavior (41). Of particular interest in cancer is the major cell adhesion protein E-cadherin, which can be shed following cleavage of extracellular domain by MMPs, although the exact isoforms involved are unknown. Current study suggests that MMP-2 and MMP-9 may be involved in the process. In fact, an 80-kDa shedding E-cadherin fragment is detectable in urine and serum of patients with variety of cancers and serum levels have been shown to correlate positively with metastatic prostate cancer and disease recurrence (42, 43). The consequence of sE-cad shedding can be manifold. For one, E-cadherin shedding causes loss of cell-to-cell adhesion and leads to epithelial-mesenchymal transition (44). In addition, β-catenin bound to cytoplasmic domain of E-cadherin is disengaged and can translocate to the nucleus with resultant effects on β-catenin–dependent transcription and cell proliferation (45).

On the other hand, the direct role of shed sE-cad fragment on cells is unclear. Using immunoprecipitation technique to remove sE-cad from media, we were unable to show difference in growth of DU145 cells either in presence or absence of sE-cad (data not shown). Whereas other studies have shown that sE-cad shedding through induction of Kallikrein 6 in skin cancer cells or treatment with hepatocyte growth factor in stomach cancer cells is associated with increased cell proliferation and motility, whether the effects are directly

Figure 6. The expression of MMP-2 positively correlates with PKD1 (PRKD1) in human prostate cancer tissue. A, box plots of PRKD1, MMP-2, and MMP-9 expression levels in benign (Ben), primary (Prim), and metastatic (Meta) human prostate cancer tissue microarray data set (GSE3325). The box represents the interquartile range of data with various samples, and the line through that box represents the median of the distribution. The range is indicated by the whiskers on the plot. Benign tumor, n = 6; primary tumor, n = 7; metastatic tumor samples, n = 6. B, scattered plots of PRKD1 versus MMP-2 and MMP-9 of all samples displayed in A. C, the heat map presentation of PRKD1, MMP-2, and MMP-9 expression in normal (Norm), primary tumor, and metastatic (Meta) prostate tissue from microarray data set GSE6919.
due to of shedding of sE-cad fragment is unclear (46, 47). Data in the literature suggest that sE-cad shedding is an association rather than causal in cellular phenotypic effects observed and that other mechanisms may be involved. However, other cleavage substrates of MMPs have been shown to affect cellular phenotype. MMPs might negatively regulate cancer cell growth by regulating proapoptotic molecules like Fas ligand (FaslL) and TNF-α, which have a tumor suppressor effect in the early phase of oncogenesis (7). The FaslL/Fas receptor (Fas) is an important mediator for apoptosis and has also been shown to be involved in apoptosis of prostate epithelial tissues. MMP-7 and MMP-3 can shed soluble FaslL, which induces apoptosis in epithelial cells through Fasl/Fas pathway (10, 41).

It is also interesting in this study that MMP-2 and MMP-9 show antiproliferative effect in PKD1-transfected cells, but not in controls, suggesting that overexpression of PKD1 is necessary for antiproliferative effects of MMP-2 and MMP-9. We have previously shown that PKD1 and E-cadherin epistematically influence cell proliferation and motility (23) and PKD1 increases E-cadherin expression (Fig. 4A). It is possible that increased E-cadherin expression provides necessary substrate for MMP-2–dependent and MMP-9–dependent sE-cad shedding. We also showed that other proteins, such as β3-catenin and heat shock protein 27, are mediators of PKD1 effect on cell proliferation (23, 30). PKD1 is also known to interact with several other proteins, of which integrins are a family of heterodimer proteins, which are also known to influence MMP secretion in cells (48).

PKD1 complexes with β3-integrin protein, which is important for activation of Erk kinase and consequently increased secretion of MMP-2 and MMP-9 in PC cells. Notably, PKD1 influences cell migration by promoting the recycling of αvβ3-integrin to form a polarized distribution of focal adhesions at the leading edge of migrating cells (19) and also promotes α1-integrin activation in T cells by regulating Rap1 activation (20). Data from this study provide in vivo evidence of active PKD1-integrin-Erk pathway in prostate cancer, which accounts for increased MMP-2 and MMP-9 secretion. The mechanistic understanding of signaling pathway in MMP-2 and MMP-9 secretion may be important in designing therapeutic strategies and biomarker evaluation in prostate cancer. To successfully transfer the knowledge gained from this study to patient care, validation in human tissue samples is critical.

In silico analysis shows a significant direct correlation between PKD1 and MMP-2 expression, but not with MMP-9. Whereas the inconsistency in the data with regard to MMP-9 expression may highlight the potential limitations of cell line studies, the expression of MMP-9 in study tissue is about half that of MMP-2, which may have resulted in false negative results. Moreover, MMP-9 expression is associated with favorable prognosis in node-negative breast cancer and inversely with liver metastasis in colorectal cancer, thereby validating the protective role of this enzyme in other cancers (49, 50). However, relationship between PKD1 and MMP-2 expression is validated in human tissues, and the tumor-suppressive effects of MMP-2 in prostate cancer are clearly novel. Further mechanistic and validation studies with large cohort are necessary to assess the clinical utility of PKD1–MMP-2 and MMP-9 expression in prostate cancer.

In summary, our study shows that PKD1 increases MMP-2 and MMP-9 secretion, resulting in decreased prostate cancer cell proliferation and increased sE-cad shedding. The β3-integrin–ERK pathway is involved in PKD1-mediated increased MMP-2 and MMP-9 secretion and expression; PKD1 correlates directly with MMP-2 secretion in human tissues, which may be potential protein targets in human prostate cancer for either biomarker development or therapeutics. The tumor-suppressive effect of MMP-2 and MMP-9 in prostate cancer is novel and provides impetus for further mechanistic studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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