Protein Kinases D2 and D3 Are Novel Growth Regulators in HCC1806 Triple-negative Breast Cancer Cells

QIN HAO, RAYMOND MCKENZIE, HUACHEN GAN and HUA TANG

Department of Biochemistry, The University of Texas Health Science Center at Tyler, Tyler, TX, U.S.A.

Abstract. *Aim: The role of protein kinase D (PKD) in the context of breast cancer cell biology is not clear. Materials and Methods: The expression of PKD isoforms was assessed in various breast cancer cell lines and PKD isoform-specific siRNAs and selective inhibitors were used to study the role of PKD in breast cancer cell growth. Results: PKD2 and PKD3 were two major isoforms expressed at the highest levels in tumorgenic HCC1806 triple-negative breast cancer cells. Silencing PKD2 or PKD3 significantly inhibited HCC1806 cell proliferation, and PKD3 silencing had a higher inhibitory effect than PKD2 silencing on cell growth and PKD-mediated signaling. HCC1806 breast cancer cells were highly responsive to PKD inhibitors but not to a general protein kinase C (PKC) inhibitor. Conclusion: We have identified PKD2 and PKD3, especially PKD3, as novel cell growth regulators in HCC1806 triple-negative breast cancer cells. Targeting PKD instead of all PKCs effectively inhibited cell proliferation in a number of breast cancer cell lines.*

The serine/threonine protein kinase D (PKD) family of kinases include PKD1 (also called protein kinase Cμ-PKCμ), PKD2 and PKD3 (PKCν) (1, 2). PKD contains a tandem repeat of zinc finger-like cysteine-rich motifs at its *N* terminus, which display high affinity for diacylglycerol or phorbol ester, a pleckstrin homology domain, and a *C*-terminal catalytic domain that shares homology with the calmodulin-dependent kinases (1, 2). Although the PKD family kinases exhibit a homologous catalytic domain, they vary with respect to their subcellular localization, expression, and regulation (1-4). PKD1 contains a high frequency of apolar amino acids, mainly alanine and proline at the *N* terminus. PKD2 has unique *N*- and *C*-terminal domains that determine its nucleocytoplasmic shuttling, activation and substrate targeting (3, 5, 6), whereas PKD3 lacks the alanine- and proline-rich regions at the *N* terminus and an autophosphorylation site at the C terminus. These findings suggest a functional difference among PKD isoforms. To date, PKD1 is the best characterized isoform of this family, and it has been implicated in cell migration, membrane trafficking, and the expression of gene sets modulated by class II histone deacetylases (HDACs) (1, 7-10). In contrast, relatively less is known about the biological functions of PKD2 and PKD3.

Breast cancer can be classified into three major subtypes, estrogen receptor (ER)-positive, epidermal growth factor receptor-2 (ErbB2/HER2)-positive, and triple-negative (ER-negative, progesterone receptor-negative, and HER2-negative/not overexpressed) breast cancer, depending on current clinical treatment strategies. Triple-negative breast cancer comprises 15-20% of all breast cancers (11, 12). Triple-negative breast cancer has distinct clinical and pathological features, and is a clinical problem because of its aggressive behavior and poor overall survival (13). Currently, targeted therapies for triple-negative breast cancer are lacking, leaving chemotherapy as the mainstay of treatment. It has been shown that PKD1 is expressed in the normal human breast cell line MCF-10A and low invasive breast cancer cell lines (MCF-7 and BT474); PKD1 does not regulate breast cancer cell growth but inhibits breast cancer cell invasion (14). Interestingly, PKD2 and PKD3 were two major PKD isoforms found in human invasive breast cancer cells and tumor samples (14). Despite these intriguing observations, the specific roles of PKD2 and PKD3 in the context of breast cancer cell biology have not yet been explored.

In this study, we have investigated the expression and function of PKD2 and PKD3 in various breast cancer cell lines and identified PKD2 and PKD3, especially PKD3, as novel cell growth regulators in HCC1806 triple-negative breast cancer cells.

Materials and Methods

Antibodies and reagents. PKD3, epidermal growth factor receptor (EGFR), and phospho-HDAC4(S246)/HDAC5(S259)/HDAC7(S155) antibodies and reagents for chemiluminescence detection were from Cell Signaling (Beverly, MA, USA). Antibodies against PKCμ/PKD1/2 (C-20), HER2/ErbB2, and vinculin were from Santa Cruz Biotechnology

Correspondence to: Hua Tang, Department of Biochemistry, The University of Texas Health Science Center at Tyler, 11937 US Highway 271, Tyler, Texas 75708, U.S.A. Tel: +1 9038777938, e-mail: hua.tang@uthct.edu

Key Words: Protein kinase D, breast cancer, triple-negative breast cancer cells.

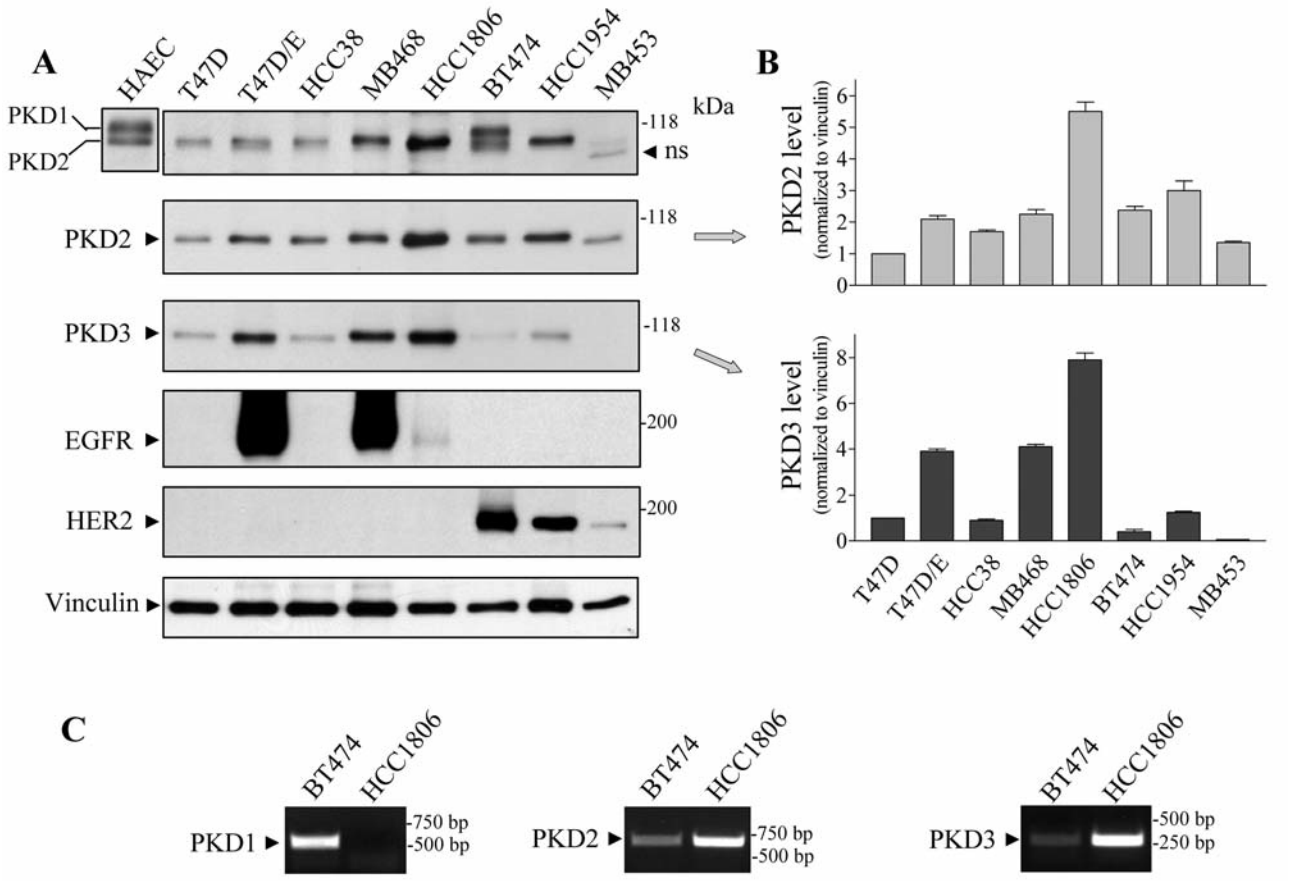

Figure 1. *Protein kinase D2 (PKD2) and PKD3 are two major PKD isoforms expressed in breast cancer cell lines. A: Cell lysates at equal protein amounts (60 μg) from different breast cancer cell lines were subjected to western blot analysis with antibodies against PKD1/2, PKD2, PKD3, epidermal growth factor receptor (EGFR), epidermal growth factor receptor-2 (HER2), or vinculin, as indicated. B: Relative expression levels of PKD2 and PKD3, after normalization to vinculin, are shown in bar graphs. C: Reverse transcription-polymerase chain reaction showing the mRNA levels of PKD1, PKD2 or PKD3 in BT474 and HCC1806 breast cancer cells. Results represent three independent experiments.*

(Santa Cruz, CA, USA). PKD2 and phospho-heat shock protein 27(S82) (HSP27-S82) antibodies were from Millipore (Billerica, MA, USA). The selective PKD inhibitor kb-NB142-70 was from Tocris Bioscience (Minneapolis, MN, USA); and Gö6976 was from LC Laboratories (Woburn, MA, USA). Gö6983 and PKCβ inhibitor were from EMD Biosciences (Billerica, MA, USA). CellTiter96 aqueous one solution reagent for cell proliferation assay was from Promega (Madison, WI, USA). Lipofectamine 2000 and Opti-MEM reduced serum medium were from Invitrogen (Carlsbad, CA, USA).

Cell culture and cell growth assay. Human breast cancer cell lines T47D, HCC38, MDA-MB-468 (MB468), HCC1806, BT474, HCC1954, and MDA-MB453 (MB453) were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum. Cells were transfected with siRNA duplexes or treated with PKD or PKC inhibitors, and the cell proliferation was assessed by CellTiter96 one solution reagent (Promega) and by cell counting with a Beckman Coulter Z1 cell counter as described previously (15).

Western blotting analysis. Western blotting was performed as described previously (16).

RNA isolation and reverse transcription polymerase chain reaction (RT-PCR). Total RNA was isolated, and semiquantitative RT-PCR was performed according to the manufacturer's protocol (Qiagen, Valencia, CA, USA) using the following primers: *PKD1* (expected product of 579 bp), 5'-TGC CAG AGC ACA TAA CGA AG-3' (forward) and 5'-TTC TCC CAC CTC AGG TCA TC-3' (reverse); *PKD2* (697 bp), 5'-CAA CCC ACA CTG CTT TGA GA-3' (forward) and 5'-CAC ACA GCT TCA CCT GAG GA-3' (reverse); *PKD3* (273 bp), 5'-CGG AGC AAA GGT TAC AAC-3' (forward) and 5'-AAG CCA AGT CTG ATA GTC CTG-3' (reverse); and internal control *GAPDH*, 5'-CGC TGA GTA CGT CGT GGA G-3'(forward) and 5'-GAG GAG TGG GTG TCG CTG TTG-3' (reverse). RT-PCR conditions were 50˚C for 30 min and 95˚C for 15 min, followed by 30 cycles of 94˚C for 30 s, 55˚C for 1 min, and 72˚C for 1 min, followed by 72˚C for 10 min.

Small-interference RNA (siRNA) and siRNA transfection. AllStars non-targeting negative control siRNA (#1027280) and the validated human *PKD2* siRNA-1 (PKD2-S1, #SI04379578), *PKD2* siRNA-2 (PKD2-S2, #SI04379571), *PKD2* siRNA-3, (PKD2-S3, #SI02224768), *PKD3* siRNA-1 (PKD3-S1, #SI02223977), *PKD3* siRNA-2 (PKD3-S2, #SI02223984), and *PKD3* siRNA-3 (PKD3-S3, #SI00301357) were

from Qiagen. *PKCα* 27-mer siRNAs and negative control siRNA were from OriGene (Rockville, MD, USA). For siRNA transfection, breast cancer cells were seeded into different plates for 24 h to reach 50-70% confluence, and then siRNA was transfected into the cells at a final concentration of 20 nM by using Lipofectamine 2000 and Opti-MEM reduced serum medium according to the manufacturer's protocols (Invitrogen). The medium was replaced with fresh growth medium 12 h after transfection. The silencing effects of siRNAs were confirmed by western blot analysis as described elsewhere (15).

Statistical analysis. Data are expressed as the mean±SE. A Student's *t*-test was used for statistical analysis. $p<0.05$ is considered statistically significant.

Results

PKD2 and PKD3 are two major PKD isoforms expressed in breast cancer cell lines. We showed that PKD1 and PKD2 were detected as 115 kDa and 105 kDa protein bands in endothelial cells by a PKD1/2 (C-20) antibody that recognizes both PKD1 and PKD2 (15). As shown in Figure 1A (*top panel*), consistent with an early report (14), a single protein band corresponding to PKD1 molecular mass (115 kDa) was only detected in BT474, a HER2-overexpressing breast cancer cell line. In contrast, a protein band corresponding to PKD2 molecular mass (105 kDa) was mainly detected in another seven breast cancer cell lines, which was confirmed by a PKD2-specific antibody (Figure 1A, *2nd panel*). Western blotting analyses revealed that the PKD2 protein was detected at an abundant level in a tumorigenic triple-negative HCC1806 breast cancer cell line (17); moderate in triple-negative HCC38 and MB468, HER2-overexpressing BT474 and HCC1954, and EGFR-overexpressing T47D (T47D/E) breast cancer cell lines; and low in ER-positive T47D and HER2-positive MB453 breast cancer cells (Figure 1B). Similar to PKD2, we found that PKD3 was also highly expressed in triple-negative HCC1806 breast cancer cell line (Figure 1A, *3rd panel*). An elevated protein level of PKD3 was also observed in MB468 and T47D/E breast cancer cells that overexpress EGFR. In contrast, the PKD3 protein level was low in HER2-positive BT474, HCC1954 and MB453 breast cancer cell lines (Figure 1B). The expression of PKD2 and PKD3, but not PKD1, was confirmed in HCC1806 breast cancer cells by RT-PCR (Figure 1C). These data indicate that PKD2 and PKD3 are two major PKD isoforms expressed in breast cancer cells and that tumorgenic HCC1806 triple-negative breast cancer cells express PKD2 and PKD3 at the highest levels among the examined breast cancer cell lines. Additionally, these data reveal that a stable expression of EGFR in T47D breast cancer cells up-regulates PKD2 and PKD3.

Identification of PKD2 and PKD3 as novel cell growth regulators in HCC1806 triple-negative breast cancer cells. Since *PKD2* and *PKD3* are highly expressed in HCC1806 triple-negative breast cancer cells, we therefore utilized the loss of function approach by silencing *PKD2* and *PKD3* with isoform-specific siRNAs to determine their roles in cell growth. As shown in Figure 2, *PKD2* or *PKD3* was markedly knocked-down by three different isoform-specific siRNAs in HCC1806 cells, and the knockdown of each isoform had only a minor effect on the expression of the other PKD isoform. Interestingly, we found that silencing *PKD2* or *PKD3* significantly inhibited HCC1806 cell growth; and *PKD3* silencing had a higher inhibitory effect (51-65% inhibition) than PKD2 silencing (25-35% inhibition) (Figure 2). In addition, we measured histone-associated DNA fragments and caspase-3 activation and found that *PKD2* or *PKD3* knockdown did not induce apoptosis of HCC1806 cells, as compared with cells transfected with control siRNA (data not shown). These novel findings indicate that PKD2 and PKD3 are critical for the proliferation of HCC1806 triple-negative breast cancer cells.

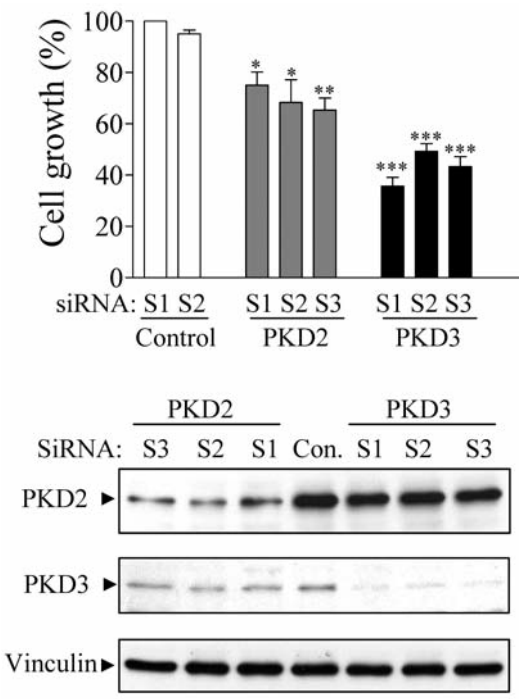

Figure 2. *Identification of protein kinase D2 (PKD2) and PKD3 as novel cell growth regulators in HCC1806 triple-negative breast cancer cells. HCC1806 cells were transfected with 20 nM non-targeting control siRNA-1 (Control-S1), control siRNA-2 (Control-S2), human PKD2 siRNA-1 (PKD2-S1), PKD2 siRNA-2 (PKD2-S2), PKD2 siRNA-3 (PKD2-S3), PKD3 siRNA-1 (PKD3-S1), PKD3 siRNA-2 (PKD3-S2), or PKD3 siRNA-3 (PKD3-S3) and grown for 100 h. Cell growth of the transfected cells was assessed by CellTiter96 aqueous one solution reagent and a Z1 cell counter. Protein levels of PKD2 and PKD3 were assessed by western blotting with specific PKD2 or PKD3 antibodies. Data are means±SEM (N=4). *p<0.05, **p<0.01 or ***p<0.001 versus control siRNA1. Results represent three independent experiments.*

We next determined the effects of *PKD2* or *PKD3* knockdown on intracellular signal transduction through

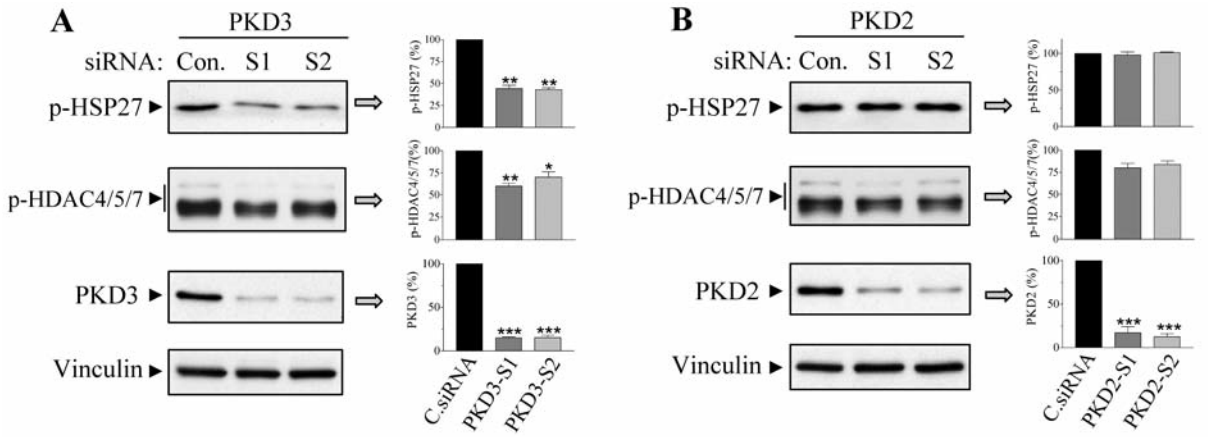

Figure 3. *Effects of protein kinase D2 (PKD2) or PKD3 knockdown on intracellular signaling in HCC1806 triple-negative breast cancer cells. HCC1806 cells were transfected with 20 nM non-targeting control (Con.) siRNA, human PKD3 siRNA-1 (PKD3-S1) or PKD3 siRNA-2 (PKD3-S2) (A); PKD2 siRNA-1 (PKD2-S1) or PKD2 siRNA-2 (PKD2-S2) (B); and grown for 96 h. Lystates (60 μg) were subjected to western blot analysis with phospho-HSP27(S82) (p-Hsp27), phospho-HDAC4(S246)/HDAC5(S259)/HDAC7(S155) (p-HDAC4/5/7), PKD2, PKD3 or vinculin antibodies as indicated. Relative changes in phosphorylation of HSP27 and HDAC4/5/7 and in protein levels of PKD2 and PKD3 are shown in bar graphs. *p<0.05, **p<0.01 or ***p<0.001 versus control siRNA. Results represent western blots of three independent experiments.*

evaluation of the phosphorylation of two well-known PKD protein substrates, such as class II HDACs (8-10) and HSP27 in HCC1806 cells. We found that *PKD3* knockdown markedly inhibited the phosphorylation of HSP27 on Ser82 and HDAC4/5/7 on Ser246/Ser259/Ser155 in HCC1806 cells (Figure 3A). In contrast, *PKD2* knockdown slightly inhibited the phosphorylation of HDAC4/5/7 on Ser246/Ser259/Ser155, and had only a minor effect on HSP27 phosphorylation (Figure 3B). These data suggest that PKD3 is an important isoform mediating the phosphorylation of PKD substrates in HCC1806 triple-negative breast cancer cells.

HCC1806 triple-negative breast cancer cells are highly responsive to PKD inhibitors. Since HCC1806 triple-negative breast cancer cells highly express PKD2 and PKD3, that are involved in cell proliferation, we examined the growth response of HCC1806 cells to PKD inhibitors compared with other breast cancer cell lines. Kb-NB142-70 is an newly-developed selective PKD inhibitor (18). As shown in Figure 4A, PKD inhibition by Kb-NB142-70 markedly and dose-dependently inhibited HCC1806 cell growth with a half maximal inhibitory concentration (IC_{50}) of 0.7 μM. In contrast, we found that HER2-overexpressing HCC1954 and BT474 breast cancer cells are less sensitive to Kb-NB142-70; and the IC_{50} for HCC1954 and BT474 cells was 2.2 or 5.5 μM, respectively. Thus, HCC1806 triple-negative breast cancer cells that express higher levels of PKD2 and PKD3 are more responsive to the PKD inhibitor Kb-NB142-70, compared to HER2-overexpressing HCC1954 and BT474 breast cancer cells.

We next utilized another PKD inhibitor, Gö6976 (19) and determined its effect on cell growth in a number of well-defined breast cancer cell lines. Gö6976 is a selective inhibitor of PKD and classical PKC isoforms (19). It inhibits PKD (IC_{50}=20 nM), PKCα (IC_{50}=2.3 nM), and PKCβI (IC_{50}=6.2 nM) *in vitro* and has been widely used as a PKD inhibitor for intact cell studies (20-22). Figure 4B shows the dose-dependent effect of Gö6976 on breast cancer cell growth. On the basis of the IC_{50} for each cell line, HCC1806 triple-negative breast cancer cells were most sensitive to Gö6976, with an IC_{50} of 65 nM. Triple-negative MB468 and HCC38, and EGFR-overexpressing T47D breast cancer cells were moderately sensitive to Gö6976, with IC_{50}s of 110-135 nM. Whereas the HER2-positive HCC1954 and MB453 breast cancer cells were less sensitive to Gö6976 with IC_{50}s of 210-400 nM, and HER2-overexpressing BT474 breast cancer cells were essentially resistant to Gö6976 (Figure 4B). Additionally, we found that a general PKC inhibitor Gö6983 (19) and a PKCβ inhibitor (23) only had minor effects on cell growth in HCC1806, HCC1954 and MB468 cells (Figure 5). Taken together, these findings reveal that the sensitivity of breast cancer cells to Gö6976 is largely correlated with PKD2 and PKD3 expression levels. It also indicates that targeting PKD and/or PKCα instead of all PKCs or PKCβ could effectively inhibit cell proliferation in breast cancer cells, especially in triple-negative breast cancer cells expressing high amounts of PKD2 and PKD3.

PKCα contributes partially to the proliferation of HCC1806 breast cancer cells. Since Gö6976 inhibits both PKD and classical PKCs (19) and PKCβ is not involved in breast cancer cell growth, we assessed whether PKCα regulates the proliferation of HCC1806 breast cancer cells. As shown in Figure 6, we knocked down *PKCα* in HCC1806 cells by using Dicer-Substrate 27-mer RNA duplexes that are

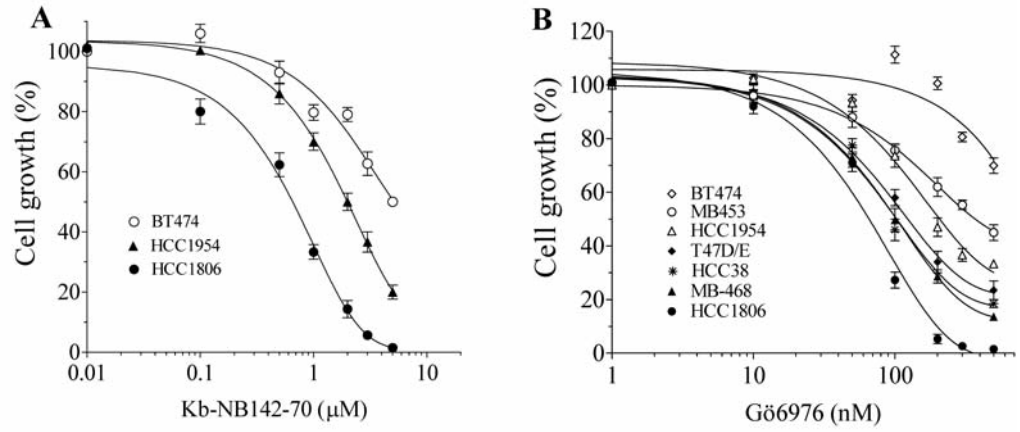

Figure 4. *Effects of protein kinase D inhibitors on cell growth in various breast cancer cell lines. Breast cancer cells were plated and allowed to attach overnight, then either a vehicle (DMSO), Kb-NB142-70 (A) or Gö6976 (B) at the indicated concentrations was added. Fresh medium and inhibitor were added every two days. After 120 h, cell growth was assessed and presented as percentage of cells treated with the same amount of vehicle. Data are means±S.E. (n=3). Similar results were obtained in three independent experiments.*

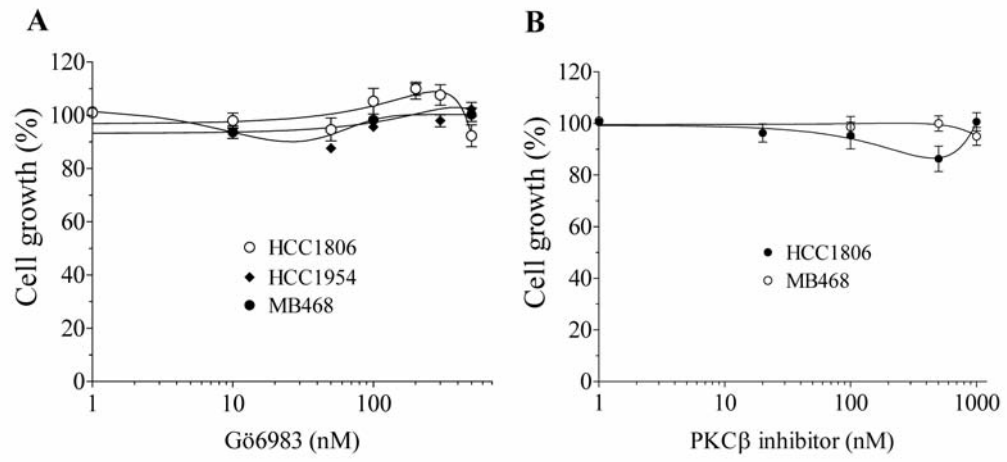

Figure 5. *Gö6983 and a protein kinase Cβ (PKCβ) inhibitor minimally affect breast cancer cell growth. HCC1806, HCC1954 and MB468 breast cancer cells were plated and allowed to attach overnight, then either a vehicle (DMSO), Gö6983 (A) or a PKCβ inhibitor (B) at the indicated concentrations was added. Fresh medium and inhibitor were added every two days. After 120 h, cell growth was assessed and presented as percentage of cells treated with same amount of vehicle. Data are means±S.E. (n=3). Similar results were obtained in three independent experiments.*

optimized for Dicer processing (24). We found that HCC1806 cell growth was only attenuated by 33% when expression of *PKCα* was knocked-down by 90% by PKCα-siRNA3, suggesting that PKCα contributes partially to the proliferation of HCC1806 breast cancer cells.

Discussion

The serine-threonine PKD family kinases include PKD1, PKD2, and PKD3 that are involved in a multitude of functions in both normal and disease states (25, 26). It has been shown that PKD2 and PKD3 are two major PKD isoforms found in human invasive breast cancer cells and tumor samples (14). However, the specific roles of PKD2 and PKD3 in the context of breast cancer cell biology are not known. In this study, we investigated the expression and function of PKD2 and PKD3 in various breast cancer cell lines and identified PKD2 and PKD3, especially PKD3, as novel cell growth regulators in HCC1806 triple-negative breast cancer cells. Our findings also reveal that targeting PKD and/or PKCα, instead of all PKCs or PKCβ could effectively inhibit cell proliferation in breast cancer cells, especially in tumorgenic HCC1806 triple-negative breast cancer cells.

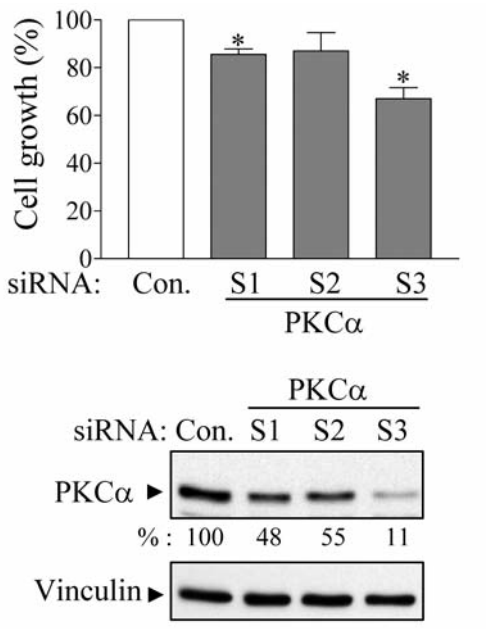

Figure 6. *Protein kinase Cα (PKCα) contributes partially to the proliferation of HCC1806 breast cancer cells. HCC1806 cells were transfected with 20 nM non-targeting control (con.) siRNA, 27-mer human PKCα siRNA-1 (PKCα-S1), PKCα siRNA-2 (PKCα-S2) or PKCα siRNA-3 (PKCα-S3) and grown for 100 h. Cell growth of the transfected cells was assessed and presented as percentage of cells treated with control siRNA. PKCα protein level was assessed by western blotting with PKCα antibody. *p<0.05 versus control siRNA. Results represent three independent experiments.*

Among the eight breast cancer cell lines with different tumor subtypes, we found that PKD1 was only detected in a HER2-overexpressing BT474 breast cancer cell line, which is consistent with an earlier report (14). In contrast, we found that PKD2 and PKD3 were expressed in all seven of the other breast cancer cell lines examined and were at the highest levels in a tumorigenic HCC1806 triple-negative breast cancer cell line. Unlike PKD2, PKD3 expression varied among breast cancer cell lines. PKD3 was highly expressed in HCC1806 and MB468 triple-negative breast cancer cell lines and in ER-positive T47D cells stably expressing EGFR. Overexpression of EGFR also increased PKD2 expression in T47D breast cancer cells. Since MB468, HCC1806 and T47D/E cells are all EGFR-positive, whether EGFR is a regulator of PKD3 expression merits further investigation. On the other hand, PKD3 expression, appeared to be low in HER2-positive BT474, HCC1954 and MB453 breast cancer cell lines.

Interestingly, we found that silencing *PKD2* or *PKD3* significantly inhibited HCC1806 cell growth without affecting cell apoptosis; and *PKD3* silencing had a higher inhibitory effect (51-65% inhibition) on cell proliferation than did *PKD2* silencing (25-35% inhibition). Furthermore, *PKD3* knockdown inhibited phosphorylation of PKD substrates HSP27 and HDAC4/5/7 to a greater extent compared with *PKD2* knockdown. These data indicate that PKD2 and PKD3, especially PKD3, is an important PKD isoform, mediating PKD signal transduction and cell proliferation in HCC1806 triple-negative breast cancer cells. It has been shown that PKD3 contributes to prostate cancer cell growth and survival (27). Importantly, PKD inhibitors Kb-NB142-70 (18) and Gö6976 (19) markedly and dose-dependently inhibited cell growth of a number of breast cancer cell lines and a pharmacological study indicated that sensitivity of the breast cancer cells to PKD inhibitors is largely correlated with the expression levels of PKD2 and PKD3. On the basis of IC_{50} for each cell line, tumorgenic HCC1806 triple-negative breast cancer cells were most sensitive to PKD inhibitors Kb-NB142-70 and Gö6976. Triple-negative MB468 and HCC38, and EGFR-overexpressing T47D breast cancer cells were also sensitive to Gö6976, whereas the HER2-overexpressing BT474, HCC1954 and MB453 breast cancer cells were less sensitive or resistant to PKD inhibitors. Triple-negative breast cancer is aggressive and confers poor overall survival; it currently lacks targeted therapies, leaving chemotherapy as the mainstay of treatment (13). Our findings suggest that PKD2 and PKD3 may be novel drug targets for therapeutic intervention in aggressive triple-negative breast cancer.

As Gö6976 inhibits both PKD and classical PKCs (19), we found that in addition to PKD, PKCα (but not PKCβ), also contributed partially to the proliferation of HCC1806 breast cancer cells. PKCα has been identified as a marker for poor prognosis of breast cancer and its expression correlates with breast cancer progression (28). Moreover, we found that Gö6983 (19), a general PKC inhibitor, had only a minor effect on cell growth in triple-negative HCC1806 and MB468 and HER2-overexpressing HCC1954 breast cancer cells. A large body of evidence indicates that the PKC family of kinases are involved in tumor development and progression. However, PKC isozymes have opposing roles in tumor biology, and clinical application of PKC inhibitors for cancer treatment, including the one of breast cancer, have resulted in disappointing outcomes (29, 30). Data from this study revealed that targeting PKD and/or PKCα instead of all PKCs or PKCβ, effectively inhibited cell proliferation in a number of breast cancer cell lines, especially in tumorigenic HCC1806 triple-negative breast cancer cells.

Acknowledgements

This work was supported in part by a departmental fund (H. T.)

Conflicts of Interest

No conflicts of interest, financial or otherwise, are declared by the Authors.

References

1 Rozengurt E, Rey O and Waldron RT: Protein kinase D signaling. J Biol Chem *280*: 13205-13208, 2005.

2 Sturany S, Van Lint J, Muller F, Wilda M, Hameister H, Hocker M, Brey A, Gern U, Vandenheede J, Gress T, Adler G and Seufferlein T: Molecular cloning and characterization of the human protein kinase D2. A novel member of the protein kinase D family of serine threonine kinases. J Biol Chem *276*: 3310-3318, 2001.

3 Auer A, von Blume J, Sturany S, von Wichert G, Van Lint J, Vandenheede J, Adler G and Seufferlein T: Role of the regulatory domain of protein kinase D2 in phorbol ester binding, catalytic activity, and nucleocytoplasmic shuttling. Mol Biol Cell *16*: 4375-4385, 2005.

4 Rey O, Yuan J, Young SH and Rozengurt E: Protein kinase C nu/protein kinase D3 nuclear localization, catalytic activation, and intracellular redistribution in response to G protein-coupled receptor agonists. J Biol Chem *278*: 23773-23785, 2003.

5 von Blume J, Knippschild U, Dequiedt F, Giamas G, Beck A, Auer A, Van Lint J, Adler G and Seufferlein T: Phosphorylation at Ser244 by CK1 determines nuclear localization and substrate targeting of PKD2. EMBO J *26*: 4619-4633, 2007.

6 Papazyan R, Rozengurt E and Rey O: The C-terminal tail of protein kinase D2 and protein kinase D3 regulates their intracellular distribution. Biochem Biophys Res Commun *342*: 685-689, 2006.

7 Eiseler T, Doppler H, Yan IK, Kitatani K, Mizuno K and Storz P: Protein kinase D1 regulates cofilin-mediated F-actin reorganization and cell motility through slingshot. Nat Cell Biol *11*: 545-556, 2009.

8 Ha CH, Wang W, Jhun BS, Wong C, Hausser A, Pfizenmaier K, McKinsey TA, Olson EN and Jin ZG: Protein kinase D-dependent phosphorylation and nuclear export of histone deacetylase 5 mediates vascular endothelial growth factor-induced gene expression and angiogenesis. J Biol Chem *283*: 14590-14599, 2008.

9 Wang S, Li X, Parra M, Verdin E, Bassel-Duby R and Olson EN: Control of endothelial cell proliferation and migration by VEGF signaling to histone deacetylase 7. Proc Natl Acad Sci USA *105*: 7738-7743, 2008.

10 Vega RB, Harrison BC, Meadows E, Roberts CR, Papst PJ, Olson EN and McKinsey TA: Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5. Mol Cell Biol *24*: 8374-8385, 2004.

11 Bauer KR, Brown M, Cress RD, Parise CA and Caggiano V: Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California Cancer Registry. Cancer *109*: 1721-1728, 2007.

12 Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS and Millikan RC: Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA *295*: 2492-2502, 2006.

13 Irvin WJ Jr. and Carey LA: What is triple-negative breast cancer? Eur J Cancer *44*: 2799-2805, 2008.

14 Eiseler T, Doppler H, Yan IK, Goodison S and Storz P: Protein kinase D1 regulates matrix metalloproteinase expression and inhibits breast cancer cell invasion. Breast Cancer Res *11*: R13, 2009.

15 Hao Q, Wang L, Zhao ZJ and Tang H: Identification of protein kinase D2 as a pivotal regulator of endothelial cell proliferation, migration, and angiogenesis. J Biol Chem *284*: 799-806, 2009.

16 Tang H, Hao Q, Rutherford SA, Low B and Zhao ZJ: Inactivation of SRC family tyrosine kinases by reactive oxygen species *in vivo*. J Biol Chem *280*: 23918-23925, 2005.

17 Finn RS, Dering J, Ginther C, Wilson CA, Glaspy P, Tchekmedyian N and Slamon DJ: Dasatinib, an orally active small molecule inhibitor of both the src and abl kinases, selectively inhibits growth of basal-type/triple-negative breast cancer cell lines growing *in vitro*. Breast Cancer Res Treat *105*: 319-326, 2007.

18 Lavalle CR, Bravo-Altamirano K, Giridhar KV, Chen J, Sharlow E, Lazo JS, Wipf P and Wang QJ: Novel protein kinase D inhibitors cause potent arrest in prostate cancer cell growth and motility. BMC Chem Biol *10*: 5, 2010.

19 Gschwendt M, Dieterich S, Rennecke J, Kittstein W, Mueller HJ and Johannes FJ: Inhibition of protein kinase C mu by various inhibitors. Differentiation from protein kinase C isoenzymes. FEBS Lett *392*: 77-80, 1996.

20 Ha CH, Jhun BS, Kao HY and Jin ZG: VEGF stimulates HDAC7 phosphorylation and cytoplasmic accumulation modulating matrix metalloproteinase expression and angiogenesis. Arterioscler Thromb Vasc Biol *28*: 1782-1788, 2008.

21 Hao Q, Wang L and Tang H: Vascular endothelial growth factor induces protein kinase D-dependent production of proinflammatory cytokines in endothelial cells. Am J Physiol Cell Physiol *296*: C821-827, 2009.

22 Park JE, Kim YI and Yi AK: Protein kinase D1: a new component in TLR9 signaling. J Immunol *181*: 2044-2055, 2008.

23 Tanaka M, Sagawa S, Hoshi J, Shimoma F, Matsuda I, Sakoda K, Sasase T, Shindo M and Inaba T: Synthesis of anilino-monoindolylmaleimides as potent and selective PKCbeta inhibitors. Bioorg Med Chem Lett *14*: 5171-5174, 2004.

24 Kim DH, Behlke MA, Rose SD, Chang MS, Choi S and Rossi JJ: Synthetic dsRNA Dicer substrates enhance RNAi potency and efficacy. Nat Biotechnol *23*: 222-226, 2005.

25 Rozengurt E: Protein kinase D signaling: multiple biological functions in health and disease. Physiology *26*: 23-33, 2011.

26 LaValle CR, George KM, Sharlow ER, Lazo JS, Wipf P and Wang QJ: Protein kinase D as a potential new target for cancer therapy. Biochim Biophys Acta *1806*: 183-192, 2010.

27 Chen J, Deng F, Singh SV and Wang QJ: Protein kinase D3 (PKD3) contributes to prostate cancer cell growth and survival through a PKCepsilon/PKD3 pathway downstream of Akt and ERK 1/2. Cancer Res *68*: 3844-3853, 2008.

28 Lonne GK, Cornmark L, Zahirovic IO, Landberg G, Jirstrom K and Larsson C: PKCalpha expression is a marker for breast cancer aggressiveness. Mol Cancer *9*: 76, 2010.

29 Bosco R, Melloni E, Celeghini C, Rimondi E, Vaccarezza M and Zauli G: Fine tuning of protein kinase C (PKC) isoforms in cancer: shortening the distance from the laboratory to the bedside. Mini Rev Med Chem *11*: 185-199, 2011.

30 Ali AS, Ali S, El-Rayes BF, Philip PA and Sarkar FH: Exploitation of protein kinase C: A useful target for cancer therapy. Cancer Treat Rev *35*: 1-8, 2009.

Received January 5, 2013
Revised January 17, 2013
Accepted January 17, 2013