Published in final edited form as:
Expert Rev Anticancer Ther. 2013 August ; 13(8): . doi:10.1586/14737140.2013.816460.

Protein Kinase D isoforms – New targets for therapy in invasive Breast Cancers?

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Summary

The Protein Kinase D (PKD) family of serine/threonine kinases consists of three members-PKD1, PKD2, and PKD3. While PKD1 in many cancers has been identified as a suppressor of the invasive phenotype, the two other PKD subtypes, PKD2 and PKD3, have been attributed oncogenic functions. In invasive Breast Cancer cells PKD1 expression is downregulated by methylation of the *PRKD1* promoter. On the other hand, PKD2 and PKD3 are not silenced, and drive proliferation, invasion, and mediate chemoresistance. Two strategies emerge to utilize this knowledge for novel treatment opportunities. First, pan PKD inhibitors could be developed and used for these aggressive cancers. An alternative approach to obtain similar effects would be to induce the re-expression of PKD1.

Keywords

Protein Kinase D; PKD; isoforms; breast cancer; therapy

Introduction

The Protein Kinase D (PKD) family of serine/threonine kinases consists of three members-PKD1, PKD2, and PKD3. In respect to some cellular pathways and functions PKD isoforms show redundancy. However, recent data suggest that in Breast Cancer PKD1 acts as a tumor suppressor and contributes to maintain the epithelial phenotype [1–3], whereas PKD2 and PKD3 initiate oncogenic functions including increased proliferation, invasiveness, and chemoresistance [4,5]. Such effects were also observed in other cancers including prostate and gastric cancers [2,6–10]. Opposite functions of members of the same kinase family generally makes it difficult to use pan inhibitors, and requires in-depth analysis of the expression patterns of these subtypes in individual tumors and then a targeted treatment strategy.

PKD isoforms and their roles in invasive breast cancer

Triple negative or basal-like breast carcinomas often show a mesenchymal phenotype. Emerging evidence suggests that PKD1 in cancer cells prevents epithelial-to-mesenchymal

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Financial Disclosure
The authors have no affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Quotes
“New therapeutic approaches are needed for invasive breast cancer”
“Protein Kinase D enzymes regulate the invasiveness of breast cancer”
“Different strategies to target PKD subtypes may be effective for invasive breast cancer”

transition (EMT) and cell motility [1,11,12]. For example, when active in normal breast cells, PKD1 phosphorylates and inactivates Snail, leading to increased expression of adhesion molecules such as E-cadherin but also prevents F-actin reorganization processes that lead to tumor cell invasion and metastasis [1–3,11–13]. Other functions of PKD1 are to decrease the expression of matrix metalloproteinases (MMPs) that previously have been linked to the invasive phenotype of triple-negative breast cancer cells [3]. Therefore, it is not surprising that activity or expression of PKD1 is downregulated in invasive epithelial cancers. For example, Heregulin (neuregulin, NRG), a ligand for the ErbB3 and ErbB4 receptors, is expressed in 30% of human breast cancer patients and correlates with poor prognosis [14] and has been shown to inhibit PKD1 [13]. Regulation of PKD1 at the gene expression level was shown for gastric and breast cancers and occurs through hypermethylation of the *PRKD1* promoter [3,9].

In contrast to PKD1, the two other isoforms PKD2 and PKD3 are upregulated in invasive breast cancer cell lines as compared to “normal” control cells. In Breast Cancer, recent data suggest that PKD2 and PKD3 have pro-tumorigenic functions. For example, it was shown that both PKD subtypes can increase breast cancer cell proliferation, migration, and drug resistance [4,5].

Interestingly, PKD1 is still expressed in less aggressive breast cancer cells that are estrogen-receptor (ER) positive. When these cells are depleted of PKD1 they become aggressive and highly motile [3]. Since the presence or absence of the PKD1 isoform seems to determine the invasiveness of cells [3], potential therapeutic strategies to target PKD isoforms are dependent on the expression status of PKD1 in the tumors. For example, estrogen-positive PKD1 expressing cells may not be targeted with pan PKD inhibitors. On the other hand, ideal targets are invasive (i.e., triple-negative) tumors which do not express PKD1 and therefore can be targeted by two strategies: chemical inhibition of PKD2 and PKD3 to block their oncogenic functions or reactivation of the silenced *PRKD1* gene leading to re-expression of PKD1. Both approaches are discussed below.

Strategy I: To inhibit PKD2 and PKD3 to block tumor growth, multi-drug resistance, and metastasis of invasive breast cancers

Targeting PKD isoforms may be most effective in triple-negative breast cancers since this subtype of cancer is difficult to treat with other strategies. In these invasive breast cancers PKD1 is downregulated [3], but PKD2 and PKD3 have been shown to promote oncogenic progression and multidrug resistance [4,5]. This makes them ideal targets for pan PKD inhibitors.

Several new small molecules targeting PKD have been recently developed. These include CRT0066101 [15], CRT5 [16], CID755673 and its analogs [17,18], 3,5-diarylazoles [19], as well as 2,6-naphthyridine and bipyridyl inhibitors and their analogs [20]. Many of these compounds show PKD-inhibiting activities *in vitro* and in cells but fail when used in whole organisms. For example, CID755673 and its derivates have been shown to effectively block prostate cancer cell proliferation, migration, and invasion [21], but get metabolized when administered to mice. So far, only CRT0066101 was successfully used in tumor cell xenografts [15]. But it is still unclear if this inhibitor actually can reach its targets *in vivo* since orthotopic animal models or animal models with spontaneous cancers have not been challenged. Consequently, so far none of these PKD inhibitors has been successfully developed for clinical use. Since the development of PKD inhibitors is a relatively new field, several other caveats are still to be tackled. For example, the specificities of most of the above compounds have not been fully elucidated, i.e., with kinome scans, and for some PKD nonspecific functions have already been described. Ideally, isoform specific inhibitors

should be available to avoid off-target effects on the other PKD subtypes. Another issue is the administration of these novel inhibitors, of which only CRT0066101 can be administered orally.

For breast cancer the use of PKD inhibitors could be effective in combination with other currently used therapies since PKD2 has been shown to mediate multidrug resistance [4]. While this strategy may be of benefit for aggressive tumors that have silenced PKD1 expression, it may not be used, for example, for estrogen-positive tumors that express PKD1 [3].

In summary, the use of pan PKD inhibitors requires detailed analysis of the tumor to target for expression of the PKD subtypes before treatment decisions are made. An alternative would be the use of isoform-specific inhibitors.

Strategy II: Re-expression and/or activation of PKD1 to block cancer metastasis

An alternative to the use of pan PKD inhibitors is the reactivation of PKD1 in invasive cancers. As mentioned above, in triple-negative breast cancer cell lines which do not express PKD1, the re-expression of PKD1 leads to a non-invasive phenotype. On the other hand, non-invasive ER-positive cells that do express PKD1 become invasive when PKD1 expression is silenced [3]. This is based on PKD1's negative regulatory effects on actin reorganization at the leading edge [3,11–13,22], but also its inhibitory function on EMT [1,2].

A reactivation strategy for PKD1 is based on the fact that in invasive breast, gastric, and other cancers the *PRKD1* gene is epigenetically silenced, whereas the expression of the two other PKD isoforms is not affected [3,9]. Re-expression can be achieved with DNA methyltransferase inhibitors, including RG108 (2-(1,3-Dioxo-1.3-dihydro-2H-isoindol-2-yl)-3-(1H-indol-3-yl)propionic acid) or the FDA-approved drug decitabine (5-aza-2′-deoxycytidine). However, DNA methyltransferase inhibitors have been shown to revert epigenetic modifications of multiple genes, including tumor suppressor genes such as *TP53* (encodes p53), or *ESR1* (encodes the estrogen receptor). Therefore, it is difficult to assess the specificity of observed effects. The clinical application of DNA methyltransferase inhibitors also raises concerns regarding reactivation of genes in normal cells, potentially leading to cancer. However, this may be of minor concern since recent studies indicate that normal cells as compared to tumor cells are less sensitive to drug-induced gene activation [23]. For example, for patients with leukemia or myelodysplatic syndrome, clinical trials with decitabine have shown promising results with few side effects [24].

Such a PKD1 re-expression strategy in invasive breast cancers may be even more effective when combined with PKD1-specific activators. So far, only few chemical activators for PKD have been described. Of these curcumin and suramin are most promising. Curcumin, a natural phenol found in tumeric, has been shown to activate PKD1 in cells [25], but a direct activation of the kinase *in vitro* was not demonstrated. Curcumin has anti-inflammatory, anti-tumor, and anti-oxidant functions, and it was shown recently that curcumin-loaded nanoparticles can accumulate within MDA-MB-231 cells and display strong anticancer properties [26]. Although curcumin has multiple targets, of which many are also implicated in PKD1 signaling, its actions may not exclusively be mediated by PKD1.

Unlike curcumin, suramin (8,8′-{Carbonylbis[imino-3,1-phenylenecarbonylimino(4-methyl-3,1-phenylene)carbonylimino]}di(1,3,5-naphthalenetrisulfonic acid) has been shown to directly activate PKC and PKD1 *in vitro* [27]. Suramin is known to inhibit the

development of tumors, angiogenesis, and tumor cell proliferation. Although PKD1 has been attributed a role in regulating all these aspects of tumor biology, it is at this point unclear if the PKC-PKD1 signaling pathway is the main or exclusive target for this compound. Suramin already has been tested in clinical trials for metastatic breast cancer [28,29], however never in combination with methyltransferase inhibitors. A question is whether such treatment would be of additional benefit or if blockage of methylation and PKD1 re-expression would be sufficient. A potential problem with using PKD1-activating compounds is that it is difficult to assess how they would affect the two other isoforms. For example, it can be predicted that in tumors where PKD1 is present, its activation is of benefit since it blocks cell motility and even may restore the epithelial phenotype, regardless if PKD2 or PKD3 are expressed. On the other hand, in tumors lacking PKD1 an activator may drive tumor progression when only PKD2 or PKD3 are expressed.

Expert commentary

In breast cancer, similar to gastric cancer and prostate cancers, PKD1 and the two other subtypes PKD2 and PKD3 have opposite functions in respect to tumor development and progression. While PKD1 maintains the epithelial phenotype and prevents cell motility, the two other isoforms have been shown to increase proliferation, invasion, and multidrug resistance of breast cancer cells.

So can PKD be a valid target in cancers, when PKD1 is acting as a tumor suppressor, whereas other PKD isoforms are oncogenic? And if yes, what is the best strategy: re-activation of PKD1 or inhibition of PKD2 and PKD3?

Generally, the use of pan PKD inhibitors may be most effective in aggressive subtypes of breast cancer which do not express PKD1. But this requires good diagnostic tools to determine the expression of different PKD subtypes. And even then, only the subset of cancers that have downregulated PKD1 expression may be targeted by such a strategy. These would include triple-negative breast cancers since in TN cells PKD1 is not expressed due to silencing of its promoter, and PKD2 and PKD3 drive tumor progression. Chemical inhibition of these two isoforms predicts a decrease in cell proliferation, cell invasiveness, and multidrug resistance. Therefore, pan PKD inhibitors in invasive cancers should have tumor antagonizing effects and also sensitize to combination therapy. While one could argue that direct inhibition of PKD2 and PKD3 may be the more specific approach, so far this strategy was not tested *in vivo* on tumors generated with TN cells. It is also unclear how pan inhibition of PKD affects the normal epithelium. Problematic also are Ductal Carcinoma In situ (DCIS) or less aggressive cancers where PKD1 is still expressed. Here inhibition of PKD1 may lead to a more aggressive phenotype.

Another option in invasive BC is to specifically re-express or reactivate PKD1. However, re-expression of PKD1 may only be obtained with relatively nonspecific approaches such as use of DNA methyltransferase inhibitors which also upregulate other genes. Ideal effects may be obtained once isoform-specific inhibitors are available to target the oncogenic members of this kinase family and combining these with the upregulation of PKD1. This may result in a phenotype reversion from an EMT cell type to an epithelial noninvasive phenotype.

Acknowledgments

This work was supported by R01-GM86435 from the NIH and a Bankhead Coley grant (1BG11) from the Florida Department of Health, all to PS. The authors would like to thanks Heike R. Döppler and Kathleen E. Norton for help with the manuscript.

References

Papers of special note have been highlighted as:

*of interest

**of considerable interest

1**. Bastea LI, Doppler H, Balogun B, Storz P. Protein kinase D1 maintains the epithelial phenotype by inducing a DNA-bound, inactive SNAI1 transcriptional repressor complex. PLoS One. 2012; 7(1):e30459. This paper shows that PKD1 promotes the epithelial phenotype in breast tissue by inhibiting EMT and motility. Their data is supported by analysis of tissue microarrays of patients. [PubMed: 22276203]

2. Du C, Zhang C, Hassan S, Biswas MH, Balaji KC. Protein kinase D1 suppresses epithelial-to-mesenchymal transition through phosphorylation of snail. Cancer Res. 2010; 70(20):7810–7819. [PubMed: 20940406]

3*. Eiseler T, Doppler H, Yan IK, Goodison S, Storz P. Protein kinase D1 regulates matrix metalloproteinase expression and inhibits breast cancer cell invasion. Breast Cancer Res. 2009; 11(1):R13. This paper described the anti-oncogenic function of PKD1 in invasive breast cancer cells and its downregulation in IDC patient samples. [PubMed: 19243594]

4. Chen J, Lu L, Feng Y, et al. PKD2 mediates multi-drug resistance in breast cancer cells through modulation of P-glycoprotein expression. Cancer Lett. 2011; 300(1):48–56. [PubMed: 20934246]

5*. Hao Q, McKenzie R, Gan H, Tang H. Protein kinases D2 and D3 are novel growth regulators in HCC1806 triple-negative breast cancer cells. Anticancer Res. 2013; 33(2):393–399. This paper shows the oncogenic functions of the PKD subtypes PKD2 and PKD3 in triple negative, invasive breast cancer cell lines. [PubMed: 23393329]

6. Azoitei N, Pusapati GV, Kleger A, et al. Protein kinase D2 is a crucial regulator of tumour cell-endothelial cell communication in gastrointestinal tumours. Gut. 2010; 59(10):1316–1330. [PubMed: 20732914]

7. Chen J, Deng F, Singh SV, 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. 2008; 68(10):3844–3853. [PubMed: 18483269]

8. Jaggi M, Rao PS, Smith DJ, et al. E-cadherin phosphorylation by protein kinase D1/protein kinase C{mu} is associated with altered cellular aggregation and motility in prostate cancer. Cancer Res. 2005; 65(2):483–492. [PubMed: 15695390]

9**. Kim M, Jang HR, Kim JH, et al. Epigenetic inactivation of protein kinase D1 in gastric cancer and its role in gastric cancer cell migration and invasion. Carcinogenesis. 2008; 29(3):629–637. First paper that demonstrated hypermethylation of the *PRKD1* promoter as a mechanism by which PKD1 expression is repressed in invasive cancer. [PubMed: 18283041]

10. Zou Z, Zeng F, Xu W, et al. PKD2 and PKD3 promote prostate cancer cell invasion by modulating NF-kappaB- and HDAC1-mediated expression and activation of uPA. J Cell Sci. 2012; 125(Pt 20):4800–4811. [PubMed: 22797919]

11. Eiseler T, Doppler H, Yan IK, Kitatani K, Mizuno K, Storz P. Protein kinase D1 regulates cofilin-mediated F-actin reorganization and cell motility through slingshot. Nat Cell Biol. 2009; 11(5): 545–556. [PubMed: 19329994]

12. Peterburs P, Heering J, Link G, Pfizenmaier K, Olayioye MA, Hausser A. Protein kinase D regulates cell migration by direct phosphorylation of the cofilin phosphatase slingshot 1 like. Cancer Res. 2009; 69(14):5634–5638. [PubMed: 19567672]

13. Doppler H, Bastea LI, Eiseler T, Storz P. Neuregulin mediates F-actin-driven cell migration through inhibition of protein kinase D1 via Rac1 protein. J Biol Chem. 2013; 288(1):455–465. [PubMed: 23148218]

14. Dunn M, Sinha P, Campbell R, et al. Co-expression of neuregulins 1, 2, 3 and 4 in human breast cancer. J Pathol. 2004; 203(2):672–680. [PubMed: 15141382]

15**. Harikumar KB, Kunnumakkara AB, Ochi N, et al. A novel small-molecule inhibitor of protein kinase D blocks pancreatic cancer growth in vitro and in vivo. Mol Cancer Ther. 2010; 9(5):

1136–1146. By using CRT0066101 in tumor xenografts they showed for the first time that chemical inhibition of PKD is effective *in vivo*. [PubMed: 20442301]

16. Evans IM, Bagherzadeh A, Charles M, et al. Characterization of the biological effects of a novel protein kinase D inhibitor in endothelial cells. Biochem J. 2010; 429(3):565–572. [PubMed: 20497126]
17. George KM, Frantz MC, Bravo-Altamirano K, et al. Design, Synthesis, and Biological Evaluation of PKD Inhibitors. Pharmaceutics. 2011; 3(2):186–228. [PubMed: 22267986]
18. Sharlow ER, Giridhar KV, LaValle CR, et al. Potent and selective disruption of protein kinase D functionality by a benzoxoloazepinolone. J Biol Chem. 2008; 283(48):33516–33526. [PubMed: 18829454]
19. Gamber GG, Meredith E, Zhu Q, et al. 3,5-diarylazoles as novel and selective inhibitors of protein kinase D. Bioorg Med Chem Lett. 2011; 21(5):1447–1451. [PubMed: 21300545]
20. Meredith EL, Ardayfio O, Beattie K, et al. Identification of orally available naphthyridine protein kinase D inhibitors. J Med Chem. 2010; 53(15):5400–5421. [PubMed: 20684591]
21. Lavalle CR, Bravo-Altamirano K, Giridhar KV, et al. Novel protein kinase D inhibitors cause potent arrest in prostate cancer cell growth and motility. BMC Chem Biol. 2010; 10:5. [PubMed: 20444281]
22. Janssens K, De Kimpe L, Balsamo M, et al. Characterization of EVL-I as a protein kinase D substrate. Cell Signal. 2009; 21(2):282–292. [PubMed: 19000756]
23. Liang G, Gonzales FA, Jones PA, Orntoft TF, Thykjaer T. Analysis of gene induction in human fibroblasts and bladder cancer cells exposed to the methylation inhibitor 5-aza-2′-deoxycytidine. Cancer Res. 2002; 62(4):961–966. [PubMed: 11861364]
24. Yang AS, Estecio MR, Garcia-Manero G, Kantarjian HM, Issa JP. Comment on “Chromosomal instability and tumors promoted by DNA hypomethylation” and “Induction of tumors in nice by genomic hypomethylation”. Science. 2003; 302(5648):1153. author reply 1153. [PubMed: 14615517]

25*. Sundram V, Chauhan SC, Ebeling M, Jaggi M. Curcumin attenuates beta-catenin signaling in prostate cancer cells through activation of protein kinase D1. PLoS One. 2012; 7(4):e35368. Shows that Protein Kinase D can be activated by curcumin. [PubMed: 22523587]

26. Yallapu MM, Othman SF, Curtis ET, et al. Curcumin-loaded magnetic nanoparticles for breast cancer therapeutics and imaging applications. Int J Nanomedicine. 2012; 7:1761–1779. [PubMed: 22619526]

27*. Gschwendt M, Kittstein W, Johannes FJ. Differential effects of suramin on protein kinase C isoenzymes. A novel tool for discriminating protein kinase C activities. FEBS Lett. 1998; 421(2): 165–168. Demonstrates that suramin is a direct activator for Protein Kinase C and Protein Kinase D enzymes. [PubMed: 9468299]

28. Gradishar WJ, Soff G, Liu J, et al. A pilot trial of suramin in metastatic breast cancer to assess antiangiogenic activity in individual patients. Oncology. 2000; 58(4):324–333. [PubMed: 10838499]
29. Lustberg MB, Pant S, Ruppert AS, et al. Phase I/II trial of non-cytotoxic suramin in combination with weekly paclitaxel in metastatic breast cancer treated with prior taxanes. Cancer Chemother Pharmacol. 2012; 70(1):49–56. [PubMed: 22729159]

Five-year view

The different PKD kinase family members provide a cellular switch preventing or promoting breast cancer. Two steps will be necessary to determine how to target PKD subtypes. First, the individual tumor needs to be carefully analyzed for*PRKD1*promoter methylation and PKD1 expression, as well as expression of the two other PKD isoforms, PKD2 and PKD3. With the recent development of monoclonal antibodies directed against all three isoforms as well as an assay system to determine methylated*PRKD1*promoter, the required tools are in place but need to be further developed for clinical use. Once this expression profile is obtained, a tailored treatment strategy could be applied. It may be difficult to develop and proof a specific reactivation therapy for PKD1, although it was shown that relatively nonspecific agents such as DNA methyltransferase inhibitors can exert effects specifically due to induction of PKD1. The best approach may be to specifically inhibit PKD2 and PKD3. Stellar progress has been made in developing pan PKD inhibitors within the last few years. Challenges now will be i) to further develop isoform-specific inhibitors, and ii) to further refine them so that they can be used*in vivo*, preferably orally. Once such inhibitors are available they may be even more effective in combination with other currently used therapeutics.

Key-issues

- PKD isoforms are differentially expressed in normal breast and aggressive BC
- PKD1 maintains the epithelial phenotype
- PKD1 is down-regulated in invasive BC
- PKD1 could be reactivated to block BC cell metastasis
- PKD2 and PKD3 promote tumor cell proliferation, invasiveness, and multidrug resistance
- PKD2 and PKD3 may be inhibited to block BC growth