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Protein kinase D enzymes – novel kinase targets in pancreatic cancer

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Summary

Pancreatic ductal adenocarcinoma (PDA) is characterized by advanced stage desmoplastic tumors with a high prevalence of genetic abnormalities. Occurrence of PDA is linked to activating Kras mutations and aberrant epidermal growth factor receptor signaling, leading to additional activation of wildtype Kras. Since Kras is difficult to target, there is a constant need to identify novel targets acting downstream of this molecule in driving the formation or progression of PDA. Recently, it was shown that Protein kinase D enzymes not only are increasingly expressed in PDA, but also causatively linked to the development and progression of this cancer. They act downstream of both mutant Kras and growth factors and therefore may represent ideal novel targets.

Keywords

Protein Kinase D; PKD; isoforms; pancreatic cancer; acinar-to-ductal metaplasia

Introduction

Pancreatic cancer has one of the highest mortality rates among all major types of cancers. The overall 5 year survival rate of pancreatic cancer patients is at approximately 5% and has not been significantly improved over decades. Approximately 95% of pancreatic cancers diagnosed are pancreatic ductal adenocarcinoma (PDA) with activating Kras mutations. Current opinion is that PDA originates from pancreatic acini which harbor oncogenic Kras mutations. These cells undergo acinar-to-ductal metaplasia (ADM), which transdifferentiates them into duct-like cells with progenitor properties that form early pancreatic intraepithelial neoplasia (PanIN) lesions. PanIN cells also acquire an upregulation of EGFR2 (HER2/neu) signaling. Eventually, after sequential acquisition of additional mutations such as inactivation of the tumor suppressor genes p53 and/or SMAD4 (late stage mutations

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Quotes

“New therapeutic targets are needed for pancreatic cancer”

“Protein Kinase D1 regulates the initiation of pancreatic ductal adenocarcinoma”

“PKD enzymes promote tumor growth, survival and invasion of PDA”

“Targeting PKD may affect tumor development and progression”

typically occurring in advanced PanIN3) PanINs further progress to pancreatic cancer (reviewed in [1]). Eventually, it was shown that in order to develop carcinoma *in situ* and PDA, pancreatic cells also need to be exposed to inflammation, which inhibits oncogene-induced senescence [2].

Protein Kinase D (PKD) enzymes are serine/threonine protein kinases that belong to the calmodulin-dependent protein kinases (CAMK) superfamily and modulate various biological processes. Mammalian PKD isoforms include PKD1, PKD2 and PKD3, which all share similar domain homology and arrangements, but also have certain distinct physiological functions. Among these isoforms, PKD1 and PKD2 have been best characterized in respect to their roles in the development and progression of PDA. For example, PKD1 has been shown to be activated downstream of both oncogenic Kras and growth factor signaling in the initiation of pancreatic cancer [3]. In addition, cholecystokinin (CCK), a signaling molecule that mediates the release of pancreatic digestive enzymes can activate PKD1 in pancreatic acini, leading to activation of NF-κB [4], a signaling pathway that may contribute to ADM and possibly upregulation of inflammatory cytokines. In PDA cell lines it was shown that PKD1 regulates many aspects of progression, including cell proliferation, survival and invasion [5,6]. In addition, a recent study demonstrated that PKD2 increases invasiveness of pancreatic cancer cells [7].

Roles of PKD isoforms in the normal pancreas

Relatively little is known on the functions of PKD enzymes in the normal pancreas. In mouse pancreas, under physiological conditions, all three PKD isoforms are present. However, expression of each isoform is associated with different types of pancreatic cells, indicating specific roles for different PKDs in executing distinct biological functions in the pancreas. PKD1 is mainly expressed in ducts [3] and islets of Langerhans. Activation of PKD1 in isolated pancreatic islets from p38δ knockout mice elevates insulin secretion and promotes pancreatic β cell survival [8]. PKD2 is expressed in acinar cells and in islets, but its functions are not characterized [3]. PKD3 is mainly expressed in acini in which it regulates amylase secretion in response to gastrointestinal hormone stimulation [3,9]. It should be noted that expression patterns of PKD isoforms in the different cell types of normal pancreas is species-specific and may be different between rodents and humans [4,10].

Roles of PKD enzymes in development and progression of PDA

Although not present in normal mouse acinar cells, PKD1 expression is upregulated in acinar cells undergoing transformation to a duct-like phenotype [3]. Such acinar-to-ductal metaplasia is a first step in PDA initiation and can be induced by growth factors (i.e. TGFα), cytokines (i.e. TNFα), chemokines (i.e. RANTES) and oncogenic Kras mutations [3,11–13]. In TGFα-induced ADM, both in 3D organoid culture of primary pancreatic acini and in pancreatic tissue from TGFα transgenic mice, only PKD1 (but not the other two isoforms PKD2 and PKD3) is detected with increased levels of protein expression and activity [3]. Similarly, elevated levels of total and active PKD1 are present in $Kras^{G12D}$-driven models for ADM and initiation of PDA. Either knockdown of PKD1 via lentivirally-delivered small

interference RNA or inhibition of PKD1 kinase activity diminishes $Kras^{G12D}$-driven ADM in an *ex vivo* 3D organoid culture system [3]. Moreover, *in vivo* an acinar cell-specific knockout of PKD1 reduces not only $Kras^{G12D}$-caused ADM, but also progression of pancreatic intraepithelial neoplasias, revealing a role of PKD1 in potentiating the establishment of high grade PanIN lesions [3]. It should be noted, that so far it is unclear if PKD1 or one of the two other isoforms drives ADM in human PDA. In pancreatic cancer cell lines the overexpression of PKD1 promotes all properties of transformation including anchorage-independent growth, invasiveness and proliferation [5,6]. Some of these effects can be mediated by neurotensin, a neuropeptide overexpressed in pancreatic cancer, which promotes cell growth of human pancreatic cancer cells by activation of a PKC-PKD1 signaling pathway [14,15]. In addition, ectopic expression of PKD1 in Panc-1 cells upregulates VEGF and IL-8 to modulate angiogenesis, which contributes to PDA progression [5]. Relatively little is known on the roles of other PKD isoforms in the development or progression of PDA, but recently it was shown that overexpression of PKD2 also increases the invasiveness of Panc89 cells through upregulation of MMP7 and MMP9 [7].

PKD inhibitors - promising new tools for treatment of PDA?

The role of PKD1 downstream of Kras and EGFR signaling provides rationale for a development of PKD inhibitors for clinical use. Several new small molecules targeting pan PKD including CRT0066101, CRT5, CID755673, and kb-NB142-70 have been recently developed [16–19]. Some of these compounds have been shown to inhibit key-events of PDA development and progression in *in vitro* cell culture, *ex vivo* 3D organoid culture and *in vivo*. For example, treatment of cultured human pancreatic cancer cells with the pan PKD inhibitor CRT0066101 induces apoptosis and reduces transformed abilities including proliferation, anchorage independent growth and invasiveness [5,18]. Inhibition of PKD1 with the pan PKD inhibitors kb-NB142-70 and CRT0066101 diminished both $Kras^{G12D}$- and TGFα-induced ADM in 3D organoid culture of primary acini, indicating that inhibition of PKD1 prevents development of PDA [3]. Moreover, in mice with human Panc-1 xenografts an oral administration of CRT0066101 abolished formation of micro blood vessel and pancreatic cancer growth [5,18].

Many compounds previously described as PKD inhibitors often show PKD-inhibiting activities *in vitro* and in cells, but, due to rapid drug metabolism in living organisms, they fail when administered in animal models [16–19,22]. So far, only CRT0066101 and CID755673 have been successfully used in animals including human pancreatic cancer xenografts [5,10,18]. Of these, CID755673 has been described to show off-target effects. So far, no off-target effects have been observed for CRT0066101. In addition, mice treated with CRT0066101 did not show any signs of distress, and no side effects on normal tissue structure of function (i.e. effects on ductal functions) were noted *in vivo* in xenograft models for pancreatic, colorectal and metastatic breast cancers [18,20,21].

None of the present PKD inhibitors so far have been successfully-developed for clinical use. Therefore, key tasks within the next five years are to further develop the use of CRT0066101, to identify novel PKD-specific inhibitors, to provide preclinical data using

animal models that also consider the host's microenvironment, and to develop them for clinical trials, either alone or in combination with current standard of care.

Summary

Although existing preclinical data provide a rationale for developing pan PKD inhibitors for clinical use to treat pancreatic cancer patients, some questions related to PKD functions in development and progression of PDA remain unanswered. For example, the initiation of PDA through acinar-to-ductal metaplasia is driven by PKD1, which acts downstream of mutant Kras and EGFR signaling pathways [3]. While it clearly was demonstrated that the other two isoforms, PKD2 and PKD3, are not involved in this initiation process, it remains to be determined whether these two isoforms are associated with progression to carcinoma *in situ*, PDA or metastasis. Therefore, it would be of great importance to assess the PKD isoforms and their functions in transgenic mice of metastatic pancreatic cancer such as $Kras^{G12D}$;$p53^{R172H}$;$Pdx1^{cre}$ (KPC) and $Kras^{G12D}$;INK4/$Arf^{-/-}$;$Pdx1^{cre}$ models which mimic metastatic disease, similar as presented in the clinic. These animal models also hold great potential for testing PKD-targeting drugs since they resemble all aspects of human pancreatic cancer including extensive stromal desmoplasia. In addition patient-derived xenograft (PDX) models could be used for testing response to treatment. However, it should be noted that PDX models typically do not show metastases, and may only be suitable for determining response of primary tumors to treatment. A key question for the use of PKD inhibitors that needs to be answered with above animal models is if inhibition of PKD leads to a halt of tumor growth and metastases, or if it also induced regression of established tumors and metastases.

In summary, so far accumulated preclinical data acquired with pancreatic cancer cells lines, genetic and orthotopic animal models, as well as patient samples suggest PKD enzymes as targets in PDA. Therefore, PKD inhibitors hold great potential as therapeutic drugs for pancreatic cancer patients. Moreover, since they may prevent ADM and inflammatory signaling, PKD inhibitors may also be beneficial as a preventive measure to the high risk populations for pancreatic cancer such as hereditary pancreatitis patients, but also for patients with acute pancreatitis.

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