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UV-activation of PKD: implications for skin cancer

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For many years cancer has largely been treated with chemotherapeutic agents that act by poisoning cellular metabolism, with the hope that more metabolically active cancer cells will be more sensitive than normal cells to the anti-metabolic effects of the poison. However, in recent years a paradigm shift has yielded more focused approaches, with the result that many of the latest approved anti-cancer drugs target specific signaling pathways and, in some instances, specific signaling enzymes that are mutated in particular cancers. Several therapies targeting lymphoid neoplasms involve monoclonal antibodies that trigger neoplastic cells to undergo apoptosis (e.g., rituximab, alemtuzumab and newer CD-tropic antibodies). Other cancer therapeutics targeting specific signaling pathways include Avastin (bevacizumab), which inhibits vascular endothelial growth factor-mediated angiogenesis, histone deacetylase inhibitors that modulate chromatin modification, and bortezomib, used to treat multiple myeloma, which inhibits the proteasome [1]. Based on the signal amplification paradigm of protein modification, the kinome has been a prime target for anti-cancer therapeutics (reviewed in [2]). For example, Gleevec (imatinib), initially developed as an inhibitor of the Bcr-Abl tyrosine kinase fusion protein (a product of the Philadelphia chromosome) expressed in most chronic myeloid leukemias [3], was the first of several tyrosine kinase inhibitors investigated for cancer treatment. Newer therapies also target receptor tyrosine kinase-ligand complexes (epidermal growth factor, platelet-derived growth factor and her-2/neu). More recently, therapeutic agents have been developed to target serine/threonine kinases (such as Raf and mammalian target of rapamycin), and these members of the kinome have become key targets of current interest for the development of cancer treatments [2].

One such serine/threonine protein kinase that has been recently identified as a potential target for cancer therapy is protein kinase D1 (PKD1), formerly known as protein kinase C (PKC)- μ . This enzyme has homologies to both the PKC family members, in the diacylglycerol/phorbol ester-binding cysteine-rich (C1) domains, and to calcium/calmodulin-dependent protein kinases, in the catalytic domain (reviewed in [4, 5]). PKD1 is the first identified member of the newly defined PKD family, which includes also PKD2 and PKD3 (also known as PKC- ν). These kinases are recruited to cellular membranes by C1 domains and activated by transphosphorylation mediated by novel PKCs and by a Src family

tyrosine kinase pathway. Activation of PKD1 also results in autophosphorylation at two serine residues and these autophosphorylated serines have been used as markers of PKD1 activation. PKD family members have been implicated in a number of important cellular functions, such as Golgi trafficking, hypertrophy, immune response, migration, invasion and survival (reviewed in [4, 5]). Importantly, an involvement of PKD1 in regulating proliferation has also been demonstrated. Thus, overexpression of PKD1 in fibroblasts increases the response to mitogens by sustaining the phosphorylation/activation of the mitogen-activated protein kinase, extracellular signal-regulated kinase-1 and 2 (ERK-1/2) (reviewed in [6]). In pancreatic cancer cells also, PKD1 has been reported to promote proliferation (reviewed in [4]), and treatment of mice with a novel PKD inhibitor inhibits tumor growth in a pancreatic cancer cell xenograft mouse model [7]. On the other hand, the role of PKD in prostate cancer cells is somewhat controversial. One group has reported that PKD1 is anti-proliferative since for example, RNA interference-mediated knockdown of PKD1 increases proliferation [8]. On the other hand, another laboratory has demonstrated that PKD inhibition inhibits prostate cancer cell proliferation [9], although the researchers advocating this latter hypothesis have proposed that PKD3 is the isoenzyme responsible [10]. Nevertheless, PKD is clearly involved in regulating prostate cancer cell function and could therefore also be a target for therapy in this cancer.

Data from other researchers and from our laboratory suggest that PKD plays a role in regulating epidermal keratinocyte proliferation and differentiation. In support of this idea, PKD levels have been shown to be upregulated in mouse epidermal carcinomas (reviewed in [11]). Furthermore, PKD co-purifies with proliferative keratinocytes isolated from intact epidermis, and inhibition of its activity has been shown to decrease DNA synthesis *in vitro* (reviewed in [11]). Our laboratory has also shown that PKD1 co(over)-expression increases the promoter activity for the basal layer marker, keratin 5, and decreases the promoter activity of involucrin, a marker of differentiation [12]. More recently, we found that overexpression of a constitutively active PKD1 construct (in which serines 738 and 742, equivalent to serines 744 and 748 in mouse PKD, are mutated to phosphorylation-mimicking glutamates) increases keratinocyte proliferation and a dominant-negative PKD1 construct (in which serines 738 and 742 are mutated to unphosphorylatable alanines) inhibits growth [13]. Jadali and Ghazizadeh [14] also reported a role for PKD1 in the proliferation observed with reentry of growth-arrested mouse keratinocytes into the cell cycle. A pro-proliferative role of PKD1 is not limited to mouse keratinocytes, as Ivanova et al. [15] have reported that RNA interference-mediated knock down of PKD1 inhibits proliferation and promotes differentiation of human keratinocytes. These results suggesting a pro-proliferative and anti-proliferative action of PKD implicate this enzyme in the development of skin cancer. Further supporting a possible role for PKD in epidermal tumorigenesis, we have observed elevated PKD levels in a human non-melanoma skin cancer, basal cell carcinoma (BCC), as well as a neoplastic mouse keratinocyte cell line [16].

The non-melanoma skin cancers (NMSCs), basal and squamous cell carcinomas, are the most common cancers in humans, possibly occurring more often than all other cancers *combined* (see statistics at the American Cancer Society website). Indeed, it is estimated that over one million new NMSCs are diagnosed each year, with an increasing incidence, and statistics for these cancers are routinely omitted from any analysis of cancer prevalence. Although these cancers are treatable, and essentially curable, by surgical removal, there is a high incidence of recurrence. In addition, a recent study suggests that patients with NMSCs also show an approximate two-fold increased risk for the development of other (epithelial) cancers [17]. In addition, surgery to remove NMSCs can be extremely disfiguring since these cancers typically occur on visible skin surfaces such as the face. Indeed, the primary risk factor for the development of NMSCs is cumulative exposure to sun, or ultraviolet (UV) light.

Recently, we showed that UV irradiation, in particular UVB (approximately 280–320 nm wavelength light), activates keratinocyte PKD1 [18]. UVB-induced PKD1 activation was demonstrated using two antibodies recognizing two different autophosphorylated serines of the enzyme as well as an *in vitro* kinase activity assay. PKD1 activation in response to UVB involved tyrosine phosphorylation mediated by a Src family kinase cascade, rather than via a protein kinase C-mediated transphosphorylation, and was downstream of UVB-elicited oxidative stress. Importantly, adenovirus-mediated overexpression of wild-type PKD1, but not mutant constructs, protected keratinocytes from UVB-induced apoptosis [18], suggesting that UVB might select for cells with higher levels of pro-proliferative PKD1. Alternatively, active PKD1 may allow survival of UV-damaged cells. This ability of PKD1 to promote survival would be beneficial in preventing excessive apoptosis with low levels of UVB exposure, causing minimal DNA damage that can be repaired. However, if PKD1 allows survival of cells that have suffered irreparable UV-induced DNA damage, these keratinocytes with DNA mutations could continue to proliferate and form skin tumors. Thus, either a pro-proliferative or pro-survival mechanism could provide a means by which PKD1 could contribute to epidermal tumorigenesis. Moreover, these results suggest that small-molecule PKD inhibitors might be a viable therapy for the treatment of non-melanoma skin cancers. Because these inhibitors can be applied topically, with minimal systemic exposure, they could potentially be used with few side effects even if PKD has important roles in other cell types.

In addition, as discussed above, PKD1 has been shown to mediate proliferative responses in many other cell types, and this enzyme has been proposed as a potential target for the development of therapies to treat multiple types of cancer (reviewed in [5]). Therefore, insight concerning its mechanisms of activation and role in tumorigenesis will be important for the therapeutic application of PKD inhibitors to cancer. In particular, PKD inhibitors have been proposed as possible novel therapies for treatment of pancreatic [4] and prostate cancer [19]. Therefore, an understanding of the role of PKD1 in the epidermis may be important in determining the possibility of epidermal side effects of systemic treatment with these agents. For example, inhibition of PKD1 in the skin could result in increased sun sensitivity, with ultraviolet light triggering massive apoptosis of the keratinocytes comprising the epidermis. Clearly, based on the data demonstrating the importance of PKD1 in regulating several cellular responses, including proliferation and survival, further investigation into this interesting enzyme seems warranted.

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