# ВОСТОЧНО ЕВРОПЕЙСКИЙ НАУЧНЫЙ ЖУРНАЛ

DOI: 10.31618/ESSA.2782-1994.2021.4.68

#4(68), 2021 часть 4

#4(68), 2020 part 4

Восточно Европейский научный журнал
(Санкт-Петербург, Россия)
Журнал зарегистрирован и издается в России
В журнале публикуются статьи по всем научным направлениям.
Журнал издается на русском, английском и польском языках.

Статьи принимаются до 30 числа каждого месяц.
Периодичность: 12 номеров в год.
Формат - А4, цветная печать
Все статьи рецензируются
Бесплатный доступ к электронной версии журнала.

Eastern European Scientific Journal
(St. Petersburg, Russia)
The journal is registered and published in Russia
The journal publishes articles on all scientific areas.
The journal is published in Russian, English and Polish.

Articles are accepted till the 30th day of each month.
Periodicity: 12 issues per year.
Format - A4, color printing
All articles are reviewed
Free access to the electronic version of journal

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1000 экземпляров.

Отпечатано в ООО «Логика+»

198320, Санкт-Петербург,

Город Красное Село,

ул. Геологическая,

д. 44, к. 1, литера А

«Восточно Европейский Научный Журнал»

Электронная почта: info@eesa-journal.com,

https://eesa-journal.com/

1000 copies.

Printed by Logika + LLC

198320, Region: St. Petersburg,

Locality: Krasnoe Selo Town,

Geologicheskaya 44 Street,

Building 1, Litera A

"East European Scientific Journal"

Email: info@eesa-journal.com,

https://eesa-journal.com/

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# МЕДИЦИНСКИЕ НАУКИ

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## PROTEIN KINASE D1: STRUCTURE, ACTIVATION, REGULATION, SUBSTRATES, AND FUNCTIONS. ROLE IN SKIN PATHOLOGY.

**Abstract.** Protein kinase D1 (PKD1) posses proproliferative and antidifferentiative functions in human and mouse keratinocytes, prodifferentive function in hTert (N/Tert-1 or N-hTERT) keratinocytes, participate also in wound-healing process in mouse epidermis. Loss of PKD2 enhanced keratinocytes-proliferative potential, while loss of PKD3 resulted in a progressive proliferation defect, loss of clonogenicity, and diminished tissue regenerative ability. This proliferation defect was correlated with upregulation of CDK4/6 inhibitor $p15^{INK4B}$ and induction of a p53-independent G1 cell-cycle arrest. Simultaneous silencing of PKD isoforms resulted in a more pronounced proliferation defect consistent with a predominant role for PKD3 in proliferating keratinocytes. There are no data concerning regulation of the PKD1, 2 and/or 3 kinase expression in skin. The mechanisms regulating PKD1 expression are merely studied only in pancreatic and prostate cancer cells. In skin pathology, the kinase is with increased expression in psoriatic lesions and basocellular carcinoma (BCC) and downregulated in head and neck spinocellular carcinoma (HNSCC). In the multistage mouse skin carcinogenesis model, the expression of PKD1 and CD34+ (cutaneous cancer stem-cell marker) are increased with increased expression of p53, p21, c-Myc, cyclin B, p-CDK1, and Cdc25A and inhibited activation of extracellular signal-regulated kinase 1/2 (ERK1/2), increased nuclear factor-kappaB (NF-κB), cyclic adenosine 3′,5′-monophosphate-responsive element-binding protein (CREB), and CCAATenhancer-binding protein (C/EBPs) activation by increased phosphorylation of c-Jun-N-terminal kinase 1/2 (JNK1/2), p38 and phosphatidylinositol 3-kinase (PI3K)/Akt and by increased downstream target gene expression, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), ornithine decarboxylase (ODC), and vascular endothelial growth factor (VEGF). The hyperplastic and inflammatory responses in PKD1-deficient mice to topical phorbol ester were significantly suppressed suggesting involvement of PKD1 in tumor promotion (and inflammation). Consistently, when subjected to two-stage chemical skin carcinogenesis protocol, these mice were resistant to papilloma formation when compared to control littermates. There are no other data for PKD1 participation in inflammatory skin process and expression in other premalignant skin diseases. Recently, hotspot-activating mutation in PKD1, resulting in an p.Glu710Asp amino-acid substitution, was detected in 73% of salivary Polymorphous low-grade adenocarcinoma (PLGA), associated with metastasis-free survival. Its increased expression is connected with late phases of malignant melanoma, associated with high metastatic potential. PKD1 participates in the pathology of inflammatory skin diseases and skin oncogenesis, but the mechanisms of regulation of its expression and action in skin in norm and in pathological processes are still unsufficient. PKD1 is upregulated merely in BCCs and in pancreatic cancer and downregulated in HNSCC, prostate, breast gastric, and colon cancers.

*Keywords: PKD1, Protein kinase D1, PRKD1, PKD2, PKD3, Inflammatory skin diseases, Skin cancer, HNC, Head and neck cancer, PKD1 review*

**Abbreviations:**

PKD1 - Protein Kinase D1
PRKD1 - PKD1 gene
PKC - Protein Kinase C
EGF - Epidermal Growth Factor
EGFR (HER1, ErbB1) - Epidermal Growth Factor Receptor
TGF-α Transforming (Tumor) Growth Factor-alpha
hTERT - Human telomerase catalytic protein subunit
MAPK – Mitogen signal Activated Protein Kinase
ERK1/2 - Extracellular signal-Rregulated Kinase 1/2
HDACs - Histone deacetylases
MMPs - Matrix metalloproteinases
TLRs - Toll-like receptors
ILs - Interleukins
COX-2 - Cyclooxygenase-2 (PGHS-2—prostaglandin H synthase)
PMDs - Potentially malignant diseases
HNC - Head and neck cancer
HNSCC - Head and neck SCC
SCC - Squamous Cell Carcinoma (Spinocellular Carcinoma)
cSCC - cutaneus SCC
oSCC - oral SCC
BCC - Basal Cell Carcinoma (Basocellular Carcinoma)
UVB - Ultraviolet type B light

**Protein kinase D**

Protein kinase D1 (PKD1), a ubiquitous serine/threonine kinase, was originally described as a novel μ isoform of the protein kinase C (PKC) family, as it shares two cysteine rich domains (C1a and C1b) that bind phorbol esters (PMA, TPA) and

diacylglycerol (DAG) as in the PKC family. Unlike other members of the PKC family, PKD1 also has a unique pleckstrin homology (PH) domain, differentiating them from other members of the PKC family, and the catalytic domain of PKD1 is most closely related to calcium calmodulindependent kinases (CaMK).[1,2,3,4]

Protein kinase D1 (PKD1) has been implicated in numerous cellular functions, including cell survival, proliferation, differentiation, migration, cell–cell adhesion, and epithelial–mesenchymal transition (EMT). PKD1 has been reported to be downregulated in advanced prostate, breast and gastric cancers, shown to play a role in tumorigenesis and metastasis, and upregulated in basocellular carcinoma (BCCs) and pancreatic cancer. Embryonic deletion of PKD1 in mice is lethal, suggesting that PKD1 plays a crucial role in development, which cannot be replaced by other PKD family members, PKD2 and PKD3 (PKCν).[1,2,4]

A large number of external signals involved in intercellular communication, including hormones, neurotransmitters, growth and developmental factors, cytokines, and bioactive lipids, bind to receptors that promote the stimulation of the isoforms of the phospholipase C (PLC) family and catalyze the hydrolysis of phosphatidylinositol 4,5-biphosphate to produce two second messengers: Ins(1,4,5)P3 (IP3), which triggers the release of $Ca^{2+}$ from internal stores, and DAG, which elicits cellular responses through classic (α, β,γ) and novel (δ, η, ε, θ) isoforms of PKC. The mechanisms by which PKC-mediated signals are propagated to critical downstream targets remain incompletely understood. PKD1 could be stimulated by phosphatidylserine micelles containing either DAG or phorbol esters in cell-free preparations, implying that PKD1 represents a novel component of the signal transduction pathways initiated by DAG in target cells. In addition, PKD1 activation was potently blocked by prior treatment with PKC inhibitors that did not directly inhibit PKD1 catalytic activity, suggesting that rapid PKD1 activation within intact cells is mediated through PKCs.[2]

**Protein kinase D structure**

Complementary DNA clones encoding human PKD (initially called novel PKCμ) and PKD from mouse were identified by two different laboratories in 1994. Subsequently, two additional mammalian protein kinases have been identified that share extensive overall homology with PKD, termed PKCν/PKD3 and consequently PKD2.[5]

Initially, PKCμ and PKCν were labeled as members of the PKC family, creating a new subgroup of the PKCs. However, their only similarity to other nPKCs' resides in a C1 domain that is homologous to the DAG-binding domain of other PKC enzymes, and they lack the C2 domain responsible for $Ca^{2+}$ sensitivity of the conventional PKC subgroup. In addition, in contrast to PKC family members, the catalytic domain of PKD shows a very low homology to the conserved kinase domain of the PKCs and displays a distinct inhibitor and substrate specificity. The NH2-terminal part of PKD contains a pleckstrin homology (PH) domain and lacks the typical autoinhibitory pseudosubstrate motif present in PKCs. These differences made it difficult to classify PKD1, 2 and 3 in the PKC family, and recently, they were classified as a novel subgroup of the calcium/calmodulin-dependent protein kinase (CAMK) family, based on sequence similarities of the kinase domain.[2,6,7,4] PKD family is stress-activated (DAG)-stimulated serine/threonine protein kinases.[5]

The regulatory domain of PKD1 exerts an inhibitory effect on the kinase activity, since the deletion of this domain leads to full activation of PKD1. Moreover, individual regions within the regulatory domain of PKD1 seems to have an inhibitory effect. PKD1 is also fully activated when both Zn fingers are deleted, and mutations in the PH domain lead to full activation of PKD1.[6]

The N-terminal regulatory portion of PKD (Figure 1) contains a tandem repeat of zinc finger-like cysteine-rich motifs (termed the cysteine-rich domain or CRD) highly homologous to domains found in DAG/phorbol ester-sensitive PKCs and in other signaling proteins regulated by DAG, including chimerins, Ras-GRP, Munc13, and DAG kinases. Accordingly, PKD binds phorbol esters with high affinity via its CRD. The individual cysteine-rich motifs of the CRD, referred to as cys1 and cys2 (Figure 1), are functionally dissimilar with the cys2 motif responsible for the majority of high affinity [3H]phorbol 12,13-dibutyrate (PMA) binding both *in vivo* and *in vitro*. As described below, the CRD plays a critical role in mediating PKD translocation to the plasma membrane and nucleus in cells challenged with a variety of stimuli and also represses the catalytic activity of the enzyme.[5]

Recent findings show that only Zn finger 2 binds phorbol dibutyrate (PDBu, PMA, and TPA) with high affinity; hence, the two Zn fingers are functionally different. Zn finger 1 (Cys1) has a specific inhibitory effect on the catalytic activity of PKD1, but seems to have minor importance in PDBu binding. In contrast, Zn finger 2 (Cys2) is not essential for the PDBu-induced activation of the kinase, but necessary for the PDBu-dependent translocation of PKD1 to the plasma membrane. Consequently, activation and translocation of PKD1 are two separate features. In PKD1-mediated protein transport pathway, DAG is required for PKD1 recruitment to the TGN by binding to the PKD1 C1a (Zn finger 1; cys1— cysteine-rich domain) domain 157 proline site.[5,8]

Interposed between the CRD and the catalytic domain, PKD also contains a PH domain. Found in many signal transduction proteins, PH domains bind to membrane lipids as well as to other proteins. PH domains have also been determined to play an autoregulatory role in some protein kinases, including PKD. Thus, PKD mutants with deletions or with single amino-acid substitutions within the PH domain are fully active, indicating that the PH domain, like the CRD, helps to maintain PKD in an inactive catalytic state.[5]

Unlike other PH domain-containing kinases, such as PKB/Akt, PKD1 does not need its PH domain for

membrane translocation, nor for Golgi localization. This is in agreement with the lack of evidence for the interaction of the PKD1 PH domain with those phosphorylated inositol lipids that are important for the recruitment of PKB to the plasma membrane.[6] PH domain is considered to be important for the regulation of PKD1 activity and for interactions with other proteins such as PKCη, PKCε, JNK (Jun N-terminal kinase), and Gβγ.[9] Comparison analysis between mouse and human amino-acid sequence of PKD1 (NCBI) has shown bigger differences in Cys 1, acidic, and PH domain, which could influence regulation of the kinase in the two species (our analysis), and results have shown similar function of PKD1 in human and mouse epidermis (see below).[9,10,11]

Even though PKD family members have similar modular structures, they do exhibit some structural variability. These structural differences may help account for the distinct functions of PKD isoforms. For example, PKD1 and PKD2, but not PKD3 possess a C-terminal autophosphorylation motif (in PKD1 S910 and in PKD2 S876) within a PDZ binding motif. In addition, both possess an N-terminal tyrosine phosphorylation motif (in PKD1 $Tyr^{95}$ and in PKD2 $Tyr^{87}$), which is phosphorylated by Src in response to oxidative stress. Src-dependent phosphorylation of PKD1/2 at this residue generates a binding site for PKCδ which facilitates activation loop phosphorylation and ultimately activation of the kinase.[12] One should bear in mind that small sequence differences could have profound regulatory or functional consequences.[6]

The recent discovery and structure determination of a novel ubiquitin-like dimerization domain in protein kinase D (PKD) has significant implications for its activation. In light of the new structural findings, the understanding of the mechanisms underlying PKD activation is critically evaluated, with particular emphasis on the role of dimerization in PKD autophosphorylation, and the provenance and recognition of the DAG that activates PKD.[13] Crystal structure of the PKD N terminus at 2.2 Å resolution containing a previously unannotated ubiquitin-like domain (ULD), which serves as a dimerization domain. A single point mutation in the dimerization interface of the ULD not only abrogated dimerization in cells but also prevented PKD activation loop phosphorylation upon DAG production.[14,13,21]

**Protein kinase D1 activation**

Subsequent studies, which aimed to define the regulatory properties of PKD within intact cells, produced multiple lines of evidence that elucidated a mechanism of PKD activation distinct from the direct stimulation of enzyme activity by DAG/phorbol ester (PMA, TPA) plus phospholipids obtained *in vitro*. Treatment of intact cells with phorbol esters, cell-permeant DAGs, or bryostatin induced a dramatic conversion of PKD from an inactive to an active form, as shown by in vitro kinase assays performed in the absence of lipid co-activators. In all these cases, PKD activation was selectively and potently blocked by cell treatment with PKC inhibitors (e.g., GFI and Ro 31–8220) that did not directly inhibit PKD catalytic activity, suggesting that PKD activation in intact cells is mediated, directly or indirectly, through PKCs. In line with this conclusion, co-transfection of PKD with active mutant forms of “novel” PKCs (PKCs δ, ε, η, θ) (and classical PKC according to the previous data) resulted in robust PKD activation in the absence of cell stimulation.[5] nPKCs, activated also by DAG and DAG analogs (phorbol esters, PMA, and TPA) in turn phosphorylate PKD1 in the activation loop. This activation occurs through the release of autoinhibition of the PH domain, and the activation loop phosphorylation is the mechanistic trigger for this process.[6] Apart from PLC–DAG–PKC-dependent activation of PKD, Gβγ subunits can activate PKD1 through direct interaction with the PH domain, thereby regulating the restructuring and function of the Golgi apparatus. A third mechanism for PKD1 activation is through caspase-mediated cleavage during the induction of apoptosis by genotoxic drugs.[6] .

A variety of regulatory peptides, including bombesin, bradykinin, endothelin, and vasopressin, or growth factors (e.g., platelet-derived growth factor) also induced PKD activation via a PKC-dependent pathway in intact fibroblasts. These results provided the first evidence, indicating the operation of a PKC/PKD-signaling cascade in response to receptor-activated pathways. Subsequently, the functioning of PKC-dependent PKD activation has been extended to and further explored in many normal cell types, including fibroblasts, intestinal and kidney epithelial cells, smooth muscle cells, cardiomyocytes, neuronal cells, osteoblasts, B and T lymphocytes, mast cells and platelets, as well as in a variety of cancer cells. These studies revealed PKD activation in response to regulatory peptides, lysophosphatidic acid, and thrombin that act through $G_q$, $G_{12}$, $G_i$, and Rho, growth factors, such as platelet-derived growth factor and insulinlike growth factor, cross-linking of B-cell receptor and – cell receptor in B and T lymphocytes, respectively, and oxidative stress. Collectively, these studies demonstrate PKC-dependent PKD activation in a broad range of biological systems, but do not exclude the possibility of PKD activation through PKC-independent mechanism(s) (Figure 2).[5]

Tumor necrosis factor α (TNF-α), doxorubicin, and other genotoxic chemotherapeutic agents also activate PKD1, but the role of PKC in these signaling pathways is unknown.[6]

Using two-dimensional tryptic phosphopeptide mapping of metabolically 32P-labeled wild type and mutant forms of PKD, two key serine residues in the PKD-activation loop, $Ser^{744}$ and $Ser^{748}$ in mouse PKD ($Ser^{738}$/$Ser^{742}$ in human — Figure 3), were identified. Whereas a PKD mutant with $Ser^{744}$ and $Ser^{748}$ altered to alanine was resistant to activation in response to cell stimulation, mutation of both sites to glutamic acid residues (to mimic phosphorylation) generated a constitutively active PKD. Single point mutants in which glutamic acid replaced $Ser^{744}$ or $Ser^{748}$ produced partly activated kinases. The properties of these mutant forms of PKD were consistent with a role of $Ser^{744}$ and $Ser^{748}$ in phosphorylation-dependent activation.[5]

Using an antibody that recognizes PKD phosphorylated at Ser[748] and a second antibody that detects predominantly PKD phosphorylated at Ser[744], PKD-activation loop phosphorylation was demonstrated in response to regulatory peptides, expression of heterotrimeric G proteins, and oxidative stress in many cell types. In line with the existence of a kinase cascade, Ser[744] and Ser[748] also become phosphorylated in kinase-deficient forms of PKD, indicating that PKD activation depends on *trans*-phosphorylation by an upstream kinase (e.g., PKC) rather than on PKD autophosphorylation. Although phosphorylation of other serine and tyrosine residues is likely to play a role in PKD regulation, it is clear that PKD phosphorylation at Ser[744] and Ser[748] is triggered by a vast array of stimuli in multiple cell types.[5]

In human, there is evidence that certain G-protein coupled receptor agonists induce a rapid/coordinate PKC-dependent increase in PKD1 phosphorylation at Ser[738]/Ser[742] that is followed by a more sustained increase in PKD1 phosphorylation at Ser[742]; Ser[742] phosphorylation during the late phase of G-protein-coupled receptor activation occurs via an autocatalytic mechanism that does not require PKC activity. This sustained GPCR-dependent mechanism for PKD1-activation loop phosphorylation (via an autocatalytic mechanism that does not require PKC activity) promotes extracellular signal-regulated kinase activation and mitogenic signaling in some cell types.[7] The activated form of PKD1 (phosphorylated at Ser[738]/Ser[742]) than autophosphorylates at Ser[910] (Ser[916] in mouse), not required for activation but rather regulating the conformation of PKD1.[6,7] The observations that PKD1-Ser[910] phosphorylation increases in the context of PKD1 activation by growth factor receptors or phorbol esters and that constitutively active forms of PKD1 (such as the PH domain-deleted or S738E/S742E-substituted mutants) display high levels of basal Ser[910] phosphorylation led to the widespread use of PKD1-Ser[910] phosphorylation as a surrogate marker of PKD1 activity, in place of more cumbersome direct enzyme activity measurements. However, there is ample evidence that these assumptions do not apply to all experimental conditions.[7]

*In vitro* and *in vivo* examined further the role of nPKCs[PKCδ, PKCη, PKCε, and (PKCθ)], as an upstream kinase in the activation loop phosphorylation of PKD. DAG also recruits, and simultaneously activates, novel PKCs to the plasma membrane, which mediates *trans*-phosphorylation

of PKD1 on Ser[744] (in mouse PKD1). DAG and PKC mediated *trans*-phosphorylation of PKD act synergistically to promote PKD catalytic activation and autophosphorylation on Ser[748], concomitant with a persistent increase in PKD catalytic activity. Furthermore, selective suppression of PKCε expression in intact cells markedly attenuated activation loop phosphorylation induced by GPCR stimulation.[2,4] In addition, it was thought that cPKCs (PKCα, PKCβ, and PKCγ) also posses the ability to activate PKD1. VEGF stimulates HDAC5 phosphorylation and nuclear export in ECs *via* a VEGF Receptor 2 (VEGFR2)-PLCγ1-PKCα-PKD- dependent pathway.[15] Our results suppose similar pathway of PKD1 activation in proliferation of normal human keratinocytes (Figure 2).[16,17]

Although activation loop phosphorylation is critical for PKD1 activation by agonists that signal via PKC, PKD1 also is activated via a PKC-independent mechanism that is not associated with (and does not require) activation loop phosphorylation in bone morphogenetic protein 2-treated MC3T3-E1 osteoblast-like cells, reactive oxygen species activated endothelial cells, and UVB (ultraviolet type B light)-treated keratinocytes. Other events (such as other posttranslation modifications or protein–protein interactions) that disrupt autoinhibitory constraints might also increase PKD1 activity via a mechanism that does not involve (or require) activation loop phosphorylation. In this regard, dextran sulfate activates PKD1 without increasing activation loop phosphorylation—and dextran sulfate is a potent agonist for both WT and S738A/S742A-substituted PKD1 enzymes. Gschwendt *et al.* (1997) speculated that dextran sulfate activates PKD1 by disrupting an intramolecular interaction between a highly acidic region in the C1–PH interdomain and basic regions elsewhere in the enzyme.[7,18]

UVB (approximately 280–320 nm wavelength light) activates keratinocyte PKD1. PKD1 activation in response to UVB involved tyrosine phosphorylation mediated by a Src family kinase cascade, rather than via a protein, and was downstream of UVB-elicited oxidative stress.[19]

The PKD1 regulatory domain contains other phosphorylation sites that are known or predicted to regulate signaling by PKD1 (Figure 3). For example, the C1A–C1B interdomain contains a cluster of autophosphorylation sites (at Ser[205], Ser[208], Ser[219], and Ser[223]) that reside in 14-3-3 consensus

binding motifs. Autophosphorylation at these sites leads to the formation of PKD1–14-3-3τ complexes, recruitment of apoptosis signal-regulated kinase 1 to the PKD1–PH domain, activation of the apoptosis signal-regulated kinase 1-JNK pathway, c-Jun phosphorylation, and induction of apoptosis in $H_2O_2$-treated endothelial cells. This seems to be a kinase-independent mechanism for PKD1 activation of the JNK-signaling pathway, because C1A–C1B interdomain autophosphorylation is not linked to gross changes in PKD1 activity; the docking interaction between 14-3-3τ and PKD1 actually decreases PKD1 catalytic activity (Figure 4). Moreover, catalytically active PKD1 phosphorylates c-Jun at N-terminal regulatory sites (that are distinct from the sites phosphorylated by JNK) and actually inhibits JNK-dependent c-Jun phosphorylation. The PKD1 C1A–C1B interdomain also contains another phosphorylation site at position 249; studies to date suggest that Ser[249] is a target for *trans*phosphorylation by PKC and that Ser[249] phosphorylation may contribute to optimal PKD1 activation by PKC (but it is not required for PKD1 activation by lipid cofactors).[7]

Stimulation of epithelial or fibroblastic cells with G-protein-coupled receptor (GPCR) agonists, including angiotensin II or bombesin-induced rapid and persistent PKD1 phosphorylation at $Ser^{203}$, a highly conserved residue located within the PKD1 N-terminal domain. Exposure to PKD or PKC family inhibitors did not prevent PKD1 phosphorylation at $Ser^{203}$, indicating that it is not mediated by autophosphorylation. In contrast, several lines of evidence indicated that the phosphorylation of PKD1 at $Ser^{203}$ is mediated by kinases of the class I PAK subfamily. Specifically, (1) exposing cells to four structurally unrelated PAK inhibitors (PF-3758309, FRAX486, FRAX597, and IPA-3) that act via different mechanisms abrogated PKD1 phosphorylation at $Ser^{203}$; (2) siRNA-mediated knockdown of PAK1 and PAK2 in IEC-18 and Swiss 3T3 cells blunted PKD1 phosphorylation at $Ser^{203}$; and (3) phosphorylation of $Ser^{203}$ was markedly increased *in vitro* when recombinant PKD1 was incubated with either PAK1 or PAK2 in the presence of ATP. PAK inhibitors did not interfere with GPCR activation-induced rapid translocation of PKD1 to the plasma membrane, but strikingly prevented the dissociation of PKD1 from the plasma membrane and blunted the phosphorylation of nuclear targets, including class Iia histone deacetylases (HDACs). The authors concluded that PAK-mediated phosphorylation of PKD1 at $Ser^{203}$ triggers its membrane dissociation and subsequent entry into the nucleus, thereby regulating the phosphorylation of PKD1 nuclear targets, including class IIa HDACs[20] and probably Snail (as a nuclear target).

Two additional phosphorylation sites have recently been identified adjacent to the autoinhibitory PH domain at positions 421 and 412 (Figure 3). $Ser^{421}$ is a target for an autophosphorylation reaction or *trans*-phosphorylation by protein kinase A. $Ser^{412}$ is phosphorylated via a PKC-dependent mechanism (and not an autophosphorylation reaction) in neonatal cardiomyocytes treated with PMA or hypertrophic agonists such phenylephrine or endothelin-1. Phosphorylation at these sites could in theory influence intramolecular interactions involving the PH domain that limit catalytic activity (or influence the PH domain-mediated mechanism that controls nuclear export of PKD1). However, mutagenesis studies to date do not link $Ser^{421}$ phosphorylation

to changes in PKD1 localization or catalytic activity; the consequences of an S412A substitution have not been examined. Finally, p38MAPK-dependent phosphorylation of PKD1 at $Ser^{397}$ and $Ser^{401}$ in the C1–PH interdomain is identified in pancreatic β-cells, where it is implicated as a mechanism that controls insulin secretion by inhibiting PKD1 activity.[7]

Recent improvements in methods for large-scale phosphoproteomics analyses have led to the identification of a large number of additional phosphorylation sites in PKD1. It is interesting to note that these phosphorylation sites map primarily to the C1A–C1B and C1B–PH interdomains. These phosphorylation "hot spots"—in unstructured regions of the enzyme that share little homology with corresponding regions of PKD2 and PKD3—might contribute to PKD1 isoform- and organelle-specific functions (Figures 1, 3).[7]

As for PKD1, it has recently been shown that PKD2 also becomes activated downstream of G-protein-coupled receptors (GPCRs), through the activation of PLCγ, which in turn activates PKCα, PKCε, or PKCη. For PKD2, three phosphorylation sites have been identified so far: $Ser^{876}$ corresponding to $Ser^{916}$ in PKD1, and $Ser^{706}$ and $Ser^{710}$ (activation loop) corresponding to $Ser^{744}$ and $Ser^{748}$ in PKD1. Very recently, it has been found that PKD3 is also activated through a DAG–PLC–PKC-dependent pathway in B cells. PKCε, PKCθ, and PKCη are candidates for the *trans*-phosphorylation of the activation loop $Ser^{731}$ and $Ser^{735}$ of PKD3 in this activation event, sinceco-transfection studies with constitutively active and kinase-dead PKC isoforms revealed PKD3-activation loop phosphorylation by these three novel PKCs. In contrast to PKD1 and PKD2, PKD3 does not seem to have an autophosphorylation site at its C terminus.[6]

In response to stimulation by a variety of biological agents such as DAG, phorbol esters, growth factors, and activators of G proteins, the novel PKCs phosphorylate PKD at two conserved serine residues in its activation loop; in PKD1 $Ser^{738}$ and $Ser^{742}$, in PKD2 $Ser^{706}$ and $Ser^{710}$, and in PKD3 $Ser^{731}$ and $Ser^{735}$ (Figure 1), leading to the activation of the kinases. In addition to activation through the canonical DAG–PKC-signaling cascade, increasing evidence suggests that activity can be achieved through several other mechanisms. For example, PKD activation has been shown to occur in response to oxidative stress, binding of G betagamma (Gβγ) proteins to the PKD family PH domain at the Golgi, as well as caspase 3-mediated proteolytic cleavage.[12]

As it was mentioned above the crystal structure of the PKD N terminus at 2.2 Å resolution containing a previously unannotated ubiquitin-like domain (ULD), which serves as a dimerization domain. A single point mutation in the dimerization interface of the ULD not only abrogated dimerization in cells but also prevented PKD activation loop phosphorylation upon DAG production. Elsner DJ *et al.* further show that the kinase domain of PKD dimerizes in a concentration-dependent manner and autophosphorylates on a single residue in its activation loop. They also provide evidence that PKD is expressed at concentrations 2 orders of magnitude below the ULD dissociation constant in mammalian cells. Like many protein kinases, PKD exists in unstimulated cells in an inactive, unphosphorylated conformation in which its regulatory domains inhibit the activity of its kinase domain. The inactive state of PKD is characterized by sequestration The authors propose a new model for PKD activation in which agonist-elicited increases in cellular DAG concentration or treatment of cells with phorbol esters results in membrane translocation of PKD,the production of DAG leads to the local accumulation of PKD at the membrane, which drives ULD-mediated

dimerization and subsequent trans-autophosphorylation of the kinase domain of $Ser^{742}$.[14,13]

Whilst early reports proposed that activation loop phosphorylation of PKD was carried out by novel PKCs, more recent studies have demonstrated PKC-independent activation loop phosphorylation of PKD *in vivo* upon Gαq-PLCβ DAG production and demonstrated its requirement for mitogenesis. These reports found that *in vivo* autophosphorylation in the PKD activation loop is primarily on $S^{742}$ and to a minor extent on $S^{738}$ which is consistent with our finding that the isolated PKD1 kinase domain undergoes autophosphorylation primarily at S742 and much less efficiently at $S^{738}$ *in vitro.* Of particular interest is the fact that we did not observe detectable amounts of doubly phosphorylated kinase domain in this experiment, which indicates that phosphorylation of one site (predominantly $S^{742}$) may preclude autophosphorylation of the other site. Furthermore, the position of the predominant autophosphorylation site $S^{742}$ corresponds to the well-established canonical phosphorylation site observed in many other kinases. Given the authors observations, and the fact that S742 does not conform to a PKC consensus motif, they think that it is unlikely that novel PKCs directly transphosphorylate PKD on $S^{742}$. However, they cannot rule out the possibility that PKCs are involved in the regulation of PKD activity either indirectly or via phosphorylation of alternative sites, including $S^{738}$, though it should be noted that this does not conform to a PKC consensus sequence either.[14]

While trans-phosphorylation by novel PKCs seems questionable, PKD may be transphosphorylated by other PKD isoforms. Previous studies have reported that PKD isoforms form hetero-dimers and that the ability to hetero-dimerize depends on the N-terminus of the protein. This is consistent with our own findings and explained by the fact that the ULD dimerization interface is identical in the three human PKD isoforms. Together, this data suggest that ULD-mediated dimerization may permit hetero-dimerization of PKD isoforms in the cell, though the identification of heterodimers from ectopic over-expression of one or more isoforms should be treated with caution. What is not known is whether PKD heterodimers are functional: trans-isoform phosphorylation would also be dependent on kinase domain hetero-dimerization, which will require further investigation. Nevertheless, the dominant-negative phenotypes previously reported may be attributable to ULD mediated heterodimerization of PKD that occurs during ectopic over-expression of a single isoform.[14]

Beyond its role in PKD activation, the authors provide evidence that the ULD stabilises the inactive, cytosolic conformation of PKD.[14]

In cells, PKD is phosphorylated on Ser-738 mainly by upstream PKCs, while Ser-742 phosphorylation can occur as a PKC-mediated event, but under certain circumstances also as an autocatalytic event. In recent paper, Cobbaut M *et al.* described the surprising observation that PKD also displays *in vitro* autocatalytic activity towards a Tyr residue in the P + 1 loop (just before the APE motif) of the activation segment - Tyr-749. They define the molecular determinants for this unusual activity and identify a Cys residue (C705 in PKD1) in the catalytic loop as of utmost importance. In oxidative stress conditions, for example, the authors could show that Abl is an upstream kinase for the P + 1 Tyr residue, and that it specifically phosphorylates the PKD2 isoform due to an isoform-specific motif preceding the Tyr residue. In cells, PKD Tyr autophosphorylation is suppressed through the association of an inhibitory factor. Since this activity is only observed *in vitro* and suppressed in a cellular context, future research efforts will need to be designated to determine under which conditions Tyr autophosphorylation may be allowed to occur in a cellular context.[21]

**Protein kinase D1 intracellular distribution**

PKD is present in the cytosol of unstimulated cells and to a lesser extent in several intracellular compartments including Golgi, mitochondria and in secretory granules, but rapidly translocates from the cytosol to different subcellular compartments in response to receptor activation. In nonstimulated cells, PKD is in a state of very low kinase catalytic activity maintained through repression mediated by the CRD (Zn finger, C domains, cys domains, and cysteine-rich domain) and PH domains. Each translocation step is associated with a particular PKD domain and involves rapid and reversible interactions (Figure 3). The first step of PKD translocation is mediated by the cys2 motif of the CRD, which binds to DAG produced at the inner leaflet of the plasma membrane as a result of PLC stimulation.[5] Production of DAG induces CRD-mediated PKD translocation from the cytosol to the plasma membrane, where novel (and classical) PKCs are also recruited in response to DAG generation. This activation occurs through the release of autoinhibition of the PH domain. The second step, i.e., reverse translocation from the plasma membrane to the cytosol, requires the PKC-dependent phosphorylation of PKD-activation loop $Ser^{744}$ and $Ser^{748}$ ($Ser^{738}/Ser^{742}$ in human), thereby stabilizing the activation loop of this enzyme in an active conformation. The activated form of PKD1 (phosphorylated at $Ser^{738}/Ser^{742}$) than autophosphorylates at $Ser^{910}$ ($Ser^{916}$ in mouse), not required for activation but rather regulating the conformation of PKD1.[6,7] The phosphorylated and activated PKD dissociates from the plasma membrane, translocates to the cytosol, and subsequently enters into the nucleus. Phosphorylated PKD is imported, via its cys2 (Zn finger — Figure 1) motif, into the nucleus, where it transiently accumulates before being exported to the cytosol through a cys1-dependent nuclear export pathway that requires the PH domain of PKD[5] or through the formation of PKD1–14-3-3τ complexes (Figure 4).

It has also been reported that cys2 and flanking sequences can directly bind to Gαq. In ontrast, the cys1 recruits PKD to the Golgi apparatus.[5]

PKD2 also undergoes reversible translocation from the cytosol to the plasma membrane in response to GPCR stimulation. The reverse translocation of

PKD2 requires PKC activity and, as in the case of PKD, can be prevented by inhibiting the translocation of PKCε. In contrast to PKD, active PKD2 remains predominantly in the cytoplasm after its plasma membrane dissociation. Unlike either PKD or PKD2, PKD3 is present in the nucleus as well as the cytoplasm of unstimulated cells. Stimuli including GPCR agonists (e.g., neurotensin) and B-cell antigen receptor engagement induce a rapid and reversible plasma membrane translocation of PKD3. Similar to PKD, but not PKD2, GPCR activation enhances the rate of PKD3 entry into the nucleus. The differences in the intracellular distribution of the different PKD isoenzymes may confer the ability to execute multiple functions at distinct subcellular locations.[5]

Production of DAG in the plasma membrane also triggers changes in localization, phosphorylation, and catalytic activation of PKD2 and PKD3. In this way, a similar mechanism of PKD family activation can potentially generate diverse physiological responses based on the differential distribution of each isoform.[5]

**Protein kinase D1: transcriptional regulation**

A small amount of data exist, connected with the stimulus, signal pathways, kinases, and/or transcriptional factors, participating in the regulation of PKD1 expression, estimated in the last 2 years.

Doppler *et al.* demonstrate that KRas-induced activation of the canonical NF-κB pathway is one mechanism of how *PRKD1* (gene of PKD1) expression is increased and identify the binding sites for NF-κB in the *PRKD1* promoter in pancreatic cancer.[22]

Luef *et al.* demonstrate for the first time that the androgen receptor/AR coactivator nuclear receptor coactivator (AR/NCOA1) complex stimulates migration of prostate cancer cells through suppression of *PRKD1* (PKD1 gene). Furthermore, *PRKD1* was negatively regulated by AR and *PRKD1* knockdown could significantly enhance the migratory potential of the two cell lines tested. A strong decrease in migration and invasion upon *NCOA1* knockdown, independently of the cell line's AR status, was observed. The authors suggest that specifically targeting NCOA1 could restore *PRKD1* expression and reduce the migratory capability of tumor cells. Inhibition of *PRKD1* reverted the reduced migratory potential caused by *NCOA1* knockdown. Immunohistochemical staining of prostate cancer patient samples revealed a strong increase in NCOA1 expression in primary tumors compared with normal prostate tissue, while no final conclusion could be drawn for *PRKD1* expression in tumor specimens. Thus, the authors' findings directly associate the AR/NCOA1 complex with *PRKD1* regulation and cellular migration and support the concept of therapeutic inhibition of NCOA1 in prostate cancer.[23]

According to Zhang *et al.*, androgen deprivation gradually upregulated PKD1 protein expression. In LNCaP cells (prostate cancer cells with less metastatic potential), the inhibition of AR by bicalutamide also upregulated PKD1 protein expression in a concentration-dependent manner. These data suggested that AR was required for the transcriptional repression of PKD1 gene expression caused by androgen stimulation in androgen-sensitive prostate cancer cells. However, the AREs (androgen response elements—two) in PKD1 promoter did not play an active role in regulating PKD1 transcription in response to androgen stimulation. The involvement of AR and an androgen-induced repressor protein prompted the authors to conduct an esiRNA (endoribonuclease-prepared siRNA pools comprised of a heterogeneous mixture of siRNAs that all target the same mRNA sequence) screen that targeted 23 AR corepressors and other related proteins. They identified Fibroblast Growth Factor Receptor substrate 2 (FRS2) as a potential repressor of androgen-induced PKD1 repression.[24]

The adaptor protein FRS2 is a major mediator of the FGFR signaling in normal and malignant cells. FGFR stimulation by FGF leads to the tyrosine phosphorylation of FRS2, which then forms a complex with Grb2 and Sos to activate the downstream Ras/Raf/MEK/ERK-signaling pathway. Androgen-sensitive LNCaP cells express low levels of FGF2, and its expression is upregulated in response to androgen stimulation. Current data support an AR-mediated indirect mechanism involving the cell-surface adaptor protein FRS2 in the repression of PKD1 by androgen-induced AR/FGFR/FRS2/Ras/Raf/MEK/ERK pathway. As a well documented prosurvival-signaling protein, PKD1 upregulation in response to androgen deprivation and anti-androgen treatment may have significant implications in therapy resistance and progression to CRPC (the more aggressive castration-resistant prostate cancer). These data did not completely exclude the potential involvement of other pathways in the regulation of PKD1 expression by androgen, because the binding of FGF to FGFR leads to the recruitment of multiple adaptor proteins, including FRS2, Grbs, Sos, and Gab1, and induces the activation of multiple downstream signaling pathways, including not only MEK/ERK, but also PI3K/Akt, PLCγ/PKC,and Stat3 pathways.[24]

Downregulation of protein kinase D1 gene (*PRKD1*), in prostate, gastric, breast, and colon cancers in humans leads to disease progression. While the down regulation of *PRKD1* by DNA methylation in gastric cancer and by nuclear β-catenin in colon cancer has been shown, the regulatory mechanisms in other cancers are unknown. Because Nickkholgh *et al.* had demonstrated that PRKD1 is the only known kinase to phosphorylate threonine 120 ($T^{120}$) of β-catenin in prostate cancer resulting in increased [decreased according to another authors, (authors' remark)] nuclear β-catenin, the authors explored the role of β-catenin in gene regulation of PRKD1. An initial chromatin immunoprecipitation (CHIP) assay identified potential-binding sites for β-catenin in and downstream of *PRKD1* promoter and sequencing confirmed recruitment of β-catenin to a 166 base pairs sequence upstream of exon 2. Co-transfection studies with *PRKD1*-promoter–reporter suggested that β-catenin represses *PRKD1* promoter. Efforts to identify transcription factors that mediate the co-repressor effects of β-catenin identified the recruitment of both MYC and its obligate heterodimer MAX to the same

binding site as β-catenin on the *PRKD1* promoter site. Moreover, treatment with MYC inhibitor rescued the co-repressor effect of β-catenin on *PRKD1* gene expression. Prostate specific knock out

of *PRKD1* in transgenic mice demonstrated increased nuclear expression of β-catenin validating the *in vitro* studies.Functional studies showed that nuclear translocation of β-catenin as a consequence of *PRKD1* downregulation, increases AR transcriptional activity with attendant downstream effects on androgen responsive genes. In silico human gene expression analysis confirmed the downregulation of *PRKD1* in metastatic prostate cancer correlated inversely with the expression of MAX, but not MYC, and positively with MXD1 [MAX dimerization protein 1, known also as MAD in mouse; MAD1; BHLHC58 (NCBI-Gen)], a competing heterodimer of MAX, suggesting that the dimerization of MAX with either MYC or MXD1 regulates *PRKD1* gene expression. At a molecular level, whereas MYC/MAX heterodimerization leads to the activation of MYC mediated transcription, the heterodimerization of MXD1/MAX leads to the inhibition of MYC activity. The study has identified a novel auto-repressive loop that perpetuates *PRKD1* downregulation through β-catenin/MYC/MAX protein complex, perpetuating down regulation of *PRKD1* leading to increased nuclear β-catenin.[25] In addition, stabilized β-catenin translocates into the nucleus, where it binds DNAbinding factors of the lymphoid enhancer-binding factor (LEF) and T-cell factor (TCF) family and activates downstream gene expression, including c-myc and cyclin D1.[26] Interestingly, the *MYC* gene has several Wnt-responsive elements and one of the upstream regulators of MYC is nuclear β-catenin, which is increased by down regulation of PRKD1,[27,25] and CKII.[28]

In addition, the function of MYC is targeted by several upstream proteins including receptor tyrosine kinase-stimulated Ras/Raf/ERK1/2 (canonical KRAS–ERK) signaling, PI3K (phosphoinositide 3-kinase)-mediated AKT-signaling pathways, the Notch pathway, in particular Notch2, acts upstream of MYC in a KrasG12D-driven mouse model of pancreatic ductal adenocarcinoma (PDAC), CKII,[27,29,28] CaMKIIδ through ERK1/2,[30] and estrogen receptor α (a key component of the mitogenic actions of estrogens in breast cancer).[31] NF-κB transactivates also c-Myc to stimulate hTERT promoter activity (evexdb.org)[32], which are both (NF-κB and hTERT) universally activated in various malignancies and is critical for maintenance of cellular immortalization.[32]

The treatment of C4-2 prostate cancer cells (more invasive derivative of LNCaP cells and with comparatively lower expression of *PRKD1*) with MYC inhibitor increased the transcriptional expression of *PRKD1* confirming the negative regulatory role of MYC on *PRKD1* expression. MYC inhibitor treatment also decreased the proliferation rate, migration, and invasive ability of the cells.[25]

**Protein kinase D1 substrates and functions**

It is emerging that the PKDs are implicated in the regulation of a remarkable array of fundamental biological processes, including signal transduction, Golgi organization, regulating the fusion of vesicles from the *trans*-Golgi network (TGN),plasma membrane directed transport, insulin secretion and survival of pancreatic β-cells, cell-survival apoptosis, proliferation, differentiation, and migration.[7]

Although the immediate downstream targets of PKD necessary for transmitting its signals have not been fully identified, putative substrates are beginning to emerge, including the neuronal protein Kidins 220, the Ras effector RIN1, cardiac HDAC5, the vanilloid receptor type 1, and troponin I.[5] By tethering or orienting protein substrates on the enzyme, docking interactions may facilitate phosphorylation of sites that do not conform to optimal consensus phosphorylation motifs (perhaps explaining the known effects of PKD1 to phosphorylate sites in c-Jun, β-catenin, cTnI, and type II phosphatidylinositol 4-phosphate kinase that do not conform to LxRxxpS/T motifs). Although similar docking interactions (although peptide substrates typically bind to a single site within the catalytic pocket, physiologically relevant protein substrates typically bind to protein kinases at both the active site and at distal docking motifs outside the catalytic cleft) that alter the pharmacologic profile of PKD1 have not yet been identified, PKD1 is a conformationally flexible enzyme that could be regulated in this manner.[7]

**1. The PKD1 involvement in fusion of transport carriers from the TGN to the plasma membrane.** In addition to being located at the plasma membrane, cytoplasm, and nucleus, a pool of PKD1 and PKD2 is situated at the Golgi complex, where it regulates the budding of secretory vesicles from the *trans*-Golgi network. Inactivation of PKD (e.g., by expression of kinase-deficient mutants of PKD) blocks fission of *trans*-Golgi network (TGN) transport carriers, inducing the appearance of long tubules filled with cargo. At the TGN, active PKD1 and PKD2 phosphorylate Golgi localized substrates, including phosphatidylinositol 4-kinase IIIb (PI4KIIIb), a key player required for fission of TGN to-plasma membrane carrier. PI4KIIIb is recruited to the TGN membrane by the small GTPase ARF and activated by PKD-mediated phosphorylation to generate PI(4)P. In this respect, the interaction of PI-4-kinase and PI-4,5-kinase with the Zn-finger region of active PKD1 could be of importance. PKD1 might act as a scaffold to recruit these enzymes to the Golgi, where they produce phosphorylated inositol lipids. Phosphatidic acid (PA) is also implicated in fusion promotion. DAG, which recruits PKD1 to the TGN through its binding to Zn finger 1, could, in a second step, become the substrate for DAG kinases that convert DAG to PA. The production of these phosphorylated inositol lipids and/or PA would lead to the formation of a vesicle, budding off from the TGN membrane.[6] The precise signal that stimulates PKD activation at the Golgi remains unclear. Recent reports proposed intracellular $Ca^{2+}$ released from internal stores in response to $G_q$-coupled receptor agonists or direct translocation of βγ subunits from the plasma membrane to the Golgi.[2]

In agreement with a role in regulating Golgi function, PKD has been implicated in secretion. For example, PKD stimulates secretion of neurotensin from the human endocrine BON cells via the PKD protein substrate Kidins220, (kinase D-interacting substrate of 220 kDa). PKD also plays a critical role in regulating angiotensin II-induced cortisol and aldosterone secretion from H295R cells, a human adrenocortical cell line. Recent studies revealed a novel p38δ-PKD pathway that regulates insulin secretion and survival of pancreatic β-cells, suggesting a critical role for PKD in the development of diabetes mellitus.[2]

**2. In addition, Kidins220 (kinase D-interacting substrate of 220 kDa),** an integral membrane protein selectively expressed in brain and neuroendocrine cells (in neurite tips and growth cones of PC12 pheochromocytoma cells), is phosphorylated by PKD1 at serine 919, which could possibly

point to a role for PKD1 in cytoskeletal reorganization. In invasive breast cancer cells, PKD1 forms a complex with cortactin and paxillin, associated with invadopodial membranes that extend into the extracellular matrix. Invadopodia are actin-containing protrusions extending into the matrix and participating in active proteolytic matrix degradation. Cortactin has been suggested to play a role in cell motility and cellular invasion, while the exact role of paxillin remains mainly unidentified. The function of cortactin might be modulated by paxillin and PKD1 in this invasion-related complex (see below) (reviews:[2,6,4]). Phosphorylation of the actin binding protein cortactin at $Ser^{298}$ byPKD1 generates a 14-3-3-binding motif and blocks cell by migration by attenuating Arp complex-driven actin polymerization.[12]

**3. Long-term activation of the c-Jun N-terminal kinase (JNK) pathway** by the epidermal growth factor (EGF) results in phosphorylation of the $Ser^{63}$ residue of c-Jun and induction of apoptosis. Although PKD1 was found to directly interact and phosphorylate c-Jun at its N-terminus, it has also been suggested to decrease apoptosis via modulation of JNK functions and suppression of c-Jun phosphorylation. The outcome of PKD1-mediated downregulation of the JNK-signaling pathway is dependent on the cell type and the stimulus.[4] For example, this pathway is activated upon exposure to hydrogen peroxide, but not ceramide or TNF-α-induced cell death. In addition, although attenuation of JNK signaling was observed following overexpression of PKD1 in HEK cells, this was not observed in A549 non-small celllung carcinoma cells. In addition, whereas PKD1-mediated inhibition of this pathway appears to play a prominent role in pancreatic cancer cell line survival, PKD1 was found to enhance apoptosis in the renal tubular epithelial cells by activating JNK. Thus, more studies are required to elucidate the contrasting outcomes of PKD1-mediated activation of the JNK pathway in different cell types.[5,6,7,33]

**4. A model for the activation of ERK through PKD1 has been proposed:** PKC-dependent activation of PKD1 induces translocation of PKD1 to the plasma membrane, where it can phosphorylate RIN1(RAS and RAB Interacting), a protein that associates with Ras and 14-3-3 proteins (also a binding partner for PKD1). Through phosphorylation of RIN1, the association with 14-3-3 could become more intense; therefore, abrogating its ability of blocking Ras/Raf-1 interaction, Ras can dissociate and is free to be activated, such that it can stimulate the Ras/Raf/MEK/ERK/ RSK pathway.[2,6,4] Active RAS has the ability to activate three major downstream effectors. These include RAF-1 (also known as c-RAF), PI3K, and RalGDS (Raslike guanine nucleotide-dissociation stimulator).[34] The stimulatory effect of PKD on GPCR-induced cell proliferation has been linked to its ability to increase the duration of the MEK/ERK/RSK pathway, leading to the accumulation of immediate gene products including c-Fos that stimulate DNA synthesis, cell-cycle progression, and cell proliferation.[5,4] Interestingly, the sustained phase of extracellular signal-regulated kinase 1/2 (ERK) activation induced by $G_q$-coupled receptor agonists in protein kinase D (PKD) —overexpressing Swiss 3T3 fibroblasts cells requires epidermal growth factor receptor (EGFR) tyrosine kinase activity. The PKD/ERK/DNA synthesis-signaling module is not confined to fibroblasts, but operates in a variety of cell types, including cancer cells harboring Ras mutations. PKD1 overexpression in these cells increases DNA synthesis, cell proliferation, and anchorage-independent proliferation and potentiates neurotensin-stimulated DNA synthesis, at least in part, via prolongation of ERK signaling.[2] PKD1-mediated phosphorylation of RIN1 at $Ser^{292}$ blocks cell migration by stimulating also the tyrosine kinase activity of Abl.[12,34]

PKD is also implicated as a mediator in stress and disease states, including human hypertrophic cardiomyopathy, the most common cause of sudden cardiac death in the young, Bcr-Abl-induced nuclear factor κB activation in human myeloid leukemia, in oxidative stress responses (including UVB), immune regulation, diabetes, angiogenesis, and cancer.[5,7,4]

**5. Regulation of histone deacetylases by PKD1**. The DNAbinding proteins, histones, and control protein expression by regulating the access of transcription factors to the DNA sequence. Orchestrated acetylation of lysine residues by histone acetyl transferase (HAT) and its deacetylation by histone deacetylase (HDAC) enzymes determine the epigenetic regulation of genes. Deacetylation of lysine residues of histones by HDACs results in a tighter chromatin structure and transcriptional repression of genes. Aberrant histone deacetylation has been found to correlate with pathological gene repression and neoplastic transformation. PKD1 phosphorylates specific residues in class IIHDACs (HDAC5 and HDAC7) leading to their association with 14-3-3 proteins in endothelial cells and other cell types. Sequestration of HDACs in the cytoplasm via 14-3-3 complex formation relieves target genes in the nucleus from HDAC repressive actions, thereby facilitating gene expression.[2] PKD1 that is activated in endothelial cells by vascular endothelial growth factor (VEGF) phosphorylates HDAC5 at $Ser^{259/498}$ and induces cell

proliferation and angiogenesis. Studies have also revealed that the phosphorylation of HDAC7 at Ser$^{178}$, Ser$^{344}$, and Ser$^{479}$ by PKD1 that complex with 14-3-3, promoting nuclear export of HDAC7 and activation of VEGF responsive angiogenic genes in endothelial cells. Expression of a signal-resistant HDAC7 mutant protein in these cells inhibited proliferation and migration in response to VEGF (vascular endothelial growth factor).[2] PKD1-mediated modulation of HDACs has also been shown to play an essential role in B-cell response and muscle formation by regulating the transcription activity of myocyte enhancer factor-2. These studies indicate a vital role for PKD1 in HDAC modulation. However, a further understanding of this pathway would help delineate the role of PKD1 in epigenetic regulation.[4] Recent reports have revealed that PKD directly phosphorylates class II histone deacetylase 5 (HDAC5), an enzyme that induces chromatin modifications and suppresses cardiac hypertrophy (Figure 5).[5,6,7]

Studies in cardiomyocytes identify stimulus-specific differences in PKD1 activation by α1-adrenergic receptors (α1-ARs) and endothelin-1 receptors, two seemingly similar $G_q$-coupled receptors. α1-ARs induce a rapid increase in PKD1 activity that is sustained for at least 1 h; the rapid and sustained phases of α1-AR-dependent PKD1 activation both require PKC activity. In contrast, endothelin-1 receptors induce a transient PKC-dependent increase in PKD1 activity that is followed by a more sustained increase in PKD1 that does not requirePKC activity. This PKC-independent mechanism for PKD1 activation may have evolved to support signaling responses at late timepoints when PKC isoforms are downregulated. Spatiotemporal differences in PKD1 activation also have been detected in adult cardiomyocytes. Phenylephrine (α1-AR agonist) and endothelin-1 act in a similar manner to induce rapid PKD1 translocation to the sarcolemma. However, the activated form of PKD1 remains stably associated with the sarcolemma only in endothelin-1-treated cardiomyocytes. In phenylephrine-treated cardiomyocytes, activated PKD1 shuttles to the nucleus, where it phosphorylates the class IIa histone deacetylase HDAC5, because HDAC5 phosphorylation creates docking sites for 14-3-3 proteins that escort HDAC5 from the nucleus, an enzyme that suppresses cardiac hypertrophy.[27] This pathway provides a mechanism to derepress pathologic gene programs that promote cardiomyocyte hypertrophy. In theory, these subtle differences in PKD1 activation by α1-AR agonists and endothelin-1 also might influence the phosphorylation of cAMP-response element-binding protein (CREB), sarcomeric proteins such as cardiac troponin I (cTnI) or cardiac myosin-binding protein C, or other cardiac PKD1 substrates that regulate contraction, influence tissue remodeling, and contribute to the pathogenesis of certain cardiomyopathies.[7] In turn, other studies demonstrated that augmented myocardial PKD activity induces cardiac troponin I (TNNI) phosphorylation at Ser$^{24}$ and cardiac myosin-binding protein C (MYBPC3) phosphorylation at Ser$^{304}$, which reduces myofilament $Ca^{2+}$ sensitivity and increases cross-bridge cycle rate, implying that altered PKD activity impacts on contractile function.[2]

**6. The cAMP-response element-binding protein (CREB)** is phosphorylated on Ser$^{133}$ by several upstream kinases. PKD has been identified as a CREB-Ser$^{133}$ kinase that contributes to cardiac remodeling. Collectively, these studies indicate that PKD transduces stress stimuli involved in pathological cardiac remodeling and suggest that it could be a novel target in heart disease.[2] Concomitantly, PKA activity can impose a block on $\alpha$1-adrenergic stimulation and suppress PKD phosphorylation. In this context, PKA may act as a counter-regulator to modulate PKD activation.

This notion has been supported by groundbreaking work showing that acute $\beta$-adrenergic stimulation and activation of PKA inhibits phosphorylation of not only PKD, but also HDAC5, highlighting its pertinent role in the induction of hypertrophy-associated genes.[35]

**7. Modulation of androgen receptor signaling by PKD1.** The androgen receptor (AR) is a ligand-dependent transcription factor that is present in many types of cells. The binding of AR to its ligand (androgen hormones) leads to the translocation of the AR–ligand complex into the nucleus, where it binds to androgen response element (ARE) regions in the DNA to trigger transcription of various downstream genes involved in cell survival and proliferation. In prostate cancer, somatic mutation in AR results in progression of tumor from an androgen-sensitive (AS) stage to an androgen-insensitive (AI) stage that is refractory to androgendepletion treatment. Recently, it was shown that PKD1 exists in a transcription complex along with AR and a promoter sequence for prostate-specific antigen (PSA) in prostate cancer cells. PKD1 negatively regulates the function of AR in prostate cancer cells, because the overexpression of wild-type PKD1 or kinase-dead PKD1 attenuates ligand-dependent AR function. Alternatively, PKD1 knockdown enhances ligand-dependent AR activity. Studies have also revealed that PKD1 interacts and phosphorylates the Ser$^{82}$ residue of heat-shock protein 27 (Hsp27, a molecule that is necessary for nuclear translocation of AR) and represses AR functions in prostate cancer cells. The AR function is also modulated by interaction with other proteins, such as β-catenin, which augments AR functions. Because PKD1 interacts with and downregulates both nuclear β-catenin and AR transcription activity, deregulated expression of PKD1 may play a critical role in the initiation and progression of prostate cancer (see below). A better understanding of how PKD1 modulates AR signaling at the molecular level will facilitate the development of new strategies for the treatment of prostate cancer.[4,36]

Luef *et al.* demonstrate for the first time that the AR/NCOA1 complex stimulates the migration of prostate cancer cells through the suppression of *PRKD1*. Furthermore, *PRKD1* was negatively regulated by AR and PRKD1 knockdown could significantly enhance the migratory potential of the two

cell lines tested. A strong decrease in migration and invasion upon NCOA1 knockdown, independently of the cell line's AR status, was observed. The authors suggest that specifically targeting NCOA1 could restore *PRKD1* expression and reduce the migratory capability of tumor cells. Inhibition of PRKD1 reverted the reduced migratory potential caused by NCOA1 knockdown. Immunohistochemical staining of prostate cancer patient samples revealed a strong increase in NCOA1 expression in primary tumors compared with normal prostate tissue, while no final conclusion could be drawn for *PRKD1* expression in tumor specimens. Thus, the authors' findings directly associate the AR/NCOA1 complex with *PRKD1* regulation and cellular migration and support the concept of therapeutic inhibition of NCOA1 in prostate cancer.[23]

**8. PKD1 modulates cell polarity and cell adhesion.** Recent studies have suggested an important role for PKD1 in maintaining cell polarity and a critical role in enhancing cell–cell adhesion and decreasing motility. Cellular polarization is critical for differentiation, proliferation, and tissue homeostasis, which are some of the vital characteristics that are lost in cancer cells. The adhesion complex formed by the E-cadherin–β-catenin complex plays a vital role in maintaining cell–cell contact. In addition, the aberrant expression and distribution of these proteins have been associated with cancer. In addition to its role in cell adhesion, β-catenin functions as a cotranscription factor with T-cell factor (TCF) and plays an important role in the Wnt-signaling pathway. Aberrant subcellular localization of β-catenin in the nucleus leads to enhanced transcription of genes such as c-myc and cyclin D1, resulting in oncogenic transformation of the cells. In prostate cancer cells, PKD1 modulates E-cadherin and β-catenin function. Studies from Sundram *et al.* and others have shown that PKD1 interacts, phosphorylates, and modulates the functions of E-cadherin, resulting in increased cell–cell adhesion and decreased cellular motility, suggesting a pivotal role of PKD1 in prostate cancer progression and metastasis. Thus, the activation of PKD1 inhibits the oncogenic signals produced by β-catenin's cotranscription factor activity. Of interest, both β-catenin and E-cadherin, such as PKD1, have been shown to be aberrantly expressed in prostate cancer (see below).[4] In addition, the treatment of cells with phorbol-12-myristate-13-acetate (PMA) induced PKD-mediated phosphorylation of Par-1 on a residue ($Ser^{400}$) that promotes Par-1 binding to 14-3-3, thereby promoting its dissociation from lateral plasma membrane and inhibiting its activity.[2] Partitioning-defective (Par) proteins are highly conserved serine–threonine kinases that play a critical role in maintaining cell polarity. The PKD1- dependent loss of Par1b from cell membranes to coordinate polarity dynamics in cells is a likely mechanism by which PKD1 helps maintain the epithelial phenotype. Cellular polarization is critical for differentiation, proliferation, and tissue homeostasis, which are some of the vital characteristics that are lost in cancer cells.[12,37,4]

Cofilin is a molecule that mediates F-actin severing, leading to the formation of free barbed ends that are needed for actin branching and leading-edge progression towards a stimulus. PKD1 has been shown to block cofilin at multiple levels, such that the pool of inactive, S3-phosphorylated cofilin accumulates. This is achieved by PKD1-mediated negative regulation of the phosphatase Slingshot 1L (SSH1), as well as phosphorylation-mediated activation of p21-activated kinase 4 (PAK4), which is an upstream kinase of the cofilin kinases LIMK1/2.[37]

Focal adhesion dynamics and filopodium formation are regulated by PKD1 through phosphorylation of phosphatidylinositol-4-phosphate 5-kinase type-1γ (PIP5K1γ) andvasodilator-stimulated phosphoprotein. [37] Focal adhesions (FAs) are highly dynamic structures that are assembled and disassembled on a continuous basis. The balance between the two processes mediates various aspects of cell behavior, ranging from cell adhesion and spreading to directed cell migration. The turnover of FAs is regulated at multiple levels and involves a variety of signaling molecules and adaptor proteins. In response to integrin engagement, a subcellular pool of protein kinase D1 (PKD1) localizes to the FAs. PKD1 affects FAs by decreasing turnover and promoting maturation, resulting in enhanced cell adhesion. The effects of PKD1 are mediated through direct phosphorylation of FA-localized phosphatidylinositol-4-phosphate 5-kinase type-l γ (PIP5Klγ) at serine residue 448. This phosphorylation occurs in response to fibronectin-RhoA signaling and leads to a decrease in PIP5Klγs' lipid kinase activity and binding affinity for Talin in HeLa, Hek293T, NIH-3T3, and MCF-7 cells. The data reveal a novel function for PKD1 as a regulator of FA dynamics and by identifying PIP5Klγ as a novel PKD1 substrate provide mechanistic insight into this process.[38]

Type I phosphatidylinositol 4-phosphate 5-kinases (PIP-5KIs; α, β, and γ) are a family of isoenzymes that produce phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2)], substrate of PI3K, using phosphatidylinositol 4-phosphate as substrate.[39] Phosphatidylinositol phosphate kinase type Iγ (PIPKIγ) binds talin and is required for focal adhesion formation in EGF-stimulated cells.[40] PIPKIγ depletion reduced PIP2 levels to ~ 40% of control and PIP3 to undetectable levels, and inhibited vinculin localizing to focal adhesions. Taken together, PIPKIγ positively regulates focal adhesion dynamics and cancer invasion, most probably through PIP2-mediated vinculin activation.[40] PIP5 Kγ 90 kDa (PIP5 Kγ90) is activated via its interaction with the cytoskeletal protein, talin. Currently, regulatory signaling pathways of talin–PIP5 Kγ90 interaction related to FA dynamics and cell motility are not well understood. Considering the presence of Akt consensus motifs in PIP5 Kγ90, Le *et al.* examined a potential link of Akt activation to talin–PIP5 Kγ90 interaction. They found that Akt phosphorylated PIP5 Kγ90 specifically at serine 555 ($S^{555}$) *in vitro* and in epidermal growth factor (EGF)-treated cells phosphoinositide 3-kinase-dependently.

Taken together, authors results suggested that Akt-mediated PIP5 Kγ90 S555 phosphorylation is a novel regulatory point for talin binding to control PIP2 level at the FAs, thereby modulating FA dynamics and cell motility in HeLa cells (human cervix adenocarcinoma).[41] Cao *et al.* found that PIPKIγ was required for the activation of signal transducer and activator of transcription 3 (STAT3) in epithelial ovarian cancer cells, indicating that STAT3 may also be engaged in the PIPKIγ- dependent aggressiveness of epithelial ovarian cancer cells.[42] Recent studies have revealed that lipid kinase type Iγ phosphatidylinositol phosphate kinase (PIPKIγ) participates in the metastasis of breast cancer and colon cancer by regulating cell migration and invasion.[43,40] Expression of PIPKIγ correlated positively with epidermal growth factor receptor (EGFR) expression, which regulates breast cancer progression and metastasis.[43]

**9. PKD1 phosphorylate the p85 regulatory subunit of PI3K** (which is inhibited—do not bind RTKs—when it is phosphorylated in the SH2 domain by PKD1.[7] PI3Ks are activated by RTKs, such as EGFR, and the catalytic subunit phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to form phosphatidylinositol 3,4,5-triphosphate (PIP3). Interaction of PIP3 with the PH (Pleckstrin Homology) domain of AKT and PDK1 results in a conformational change causing phosphorylation of AKT/PKB by PDK1 and mammalian target of rapamycin complex 2 (mTORC2). This activates AKT that then phosphorylates proteins involved in cell growth and survival. mTOR is a protein kinase that acts downstream of PI3K and AKT and plays an important role in cell growth, survival, and protein synthesis regulation. There are two mTOR complexes: mTORC1 activates ribosomal protein S6 kinase 1 (p70S6K, which directs the translation of cell-cycle regulatory proteins such as Cyclin D1 and myc,[44] TNF-α/mTOR/S6K1 pathway activates Gli1,[45] and inactivates eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), resulting in protein translation and cell growth, whereas mTORC2 together with PDK1 phosphorylates and activates AKT.[46]

mTOR gene expression was significantly increased in psoriasis, allergic contact dermatitis (ACD), atopic dermatitis (AD) as well as in EGFR-inhibitor-induced cutaneous rash, compared to healthy skin. Psoriasis mTOR expression was also enhanced in non-lesional skin of psoriatic patients but only weakly expressed in the epidermis from healthy individuals. Conversely, mTOR gene expression resulted decreased in peripheral blood mononuclear cells (PBMC) isolated from psoriatic subjects when compared to healthy controls. To confirm the effective enhancement of mTOR in these skin inflammatory diseases, Balato *et al.* also investigated the expression of mRNA levels of key negative upstream regulators of mTOR such as TSC 1 and TSC2. TSC I and TSC2 gene expressions were not enhanced in psoriasis and ACD and significantly reduced in AD compared to healthy skin; particularly, as regards EGFR-inhibitor induced cutaneous rash, TSC1 levels were not significantly increased, whereas TSC2 gene expression was reduced. The results found by investigating TSC1 and TSC2 expressions suggest that the augmentation of mTOR in psoriasis, AD as well as ACD could be functionally active, because not inhibited by its major negative upstream regulators. Since their experiments showed that mTOR may be involved in skin inflammation, they also investigated gene expression of its major downstream effectors such as 4EBPl and S6Kl, which are known as the best output of mTORC1. Neither 4EBPI nor S6KI were significantly augmented in psoriasis, AD, ACD, or EGFR-inhibitor-induced cutaneous rash, suggesting that mTOR pathways involved in skin inflammation are different from those traditional and well known such as those implicated in protein and lipid synthesis.[47] In addition, Balato *et al.* *in vivo* and *in vitro* experiments showed lack of a significant relationship between mTOR, TNF-α, and IL-l7A, supporting the hypothesis of alternative signaling pathways for mTOR activation in skin inflammation. Taken together, their results suggested that mTOR is involved in cutaneous inflammatory process, but through a signaling not directly dependent from Th1–Th17 pathway.[47]

Activation of PI3K/Akt/mTOR pathway is a central event in many types of cancer[48] and represents a promising target for new treatment strategies. mTOR pathway is frequently activated, independent from activation of EGFR or the presence of mutant p53, particularly in HPV-positive tumors.[46] Although the PI3K/mTOR pathway is frequently altered in HNSCC (PI3K—34% amplifications and mutations in HPV(−) and the same alterations in 56% in HPV(+) HNSCC[49]), the disease has modest clinical response rates to PI3K/mTOR inhibitors. PI3K/mTOR inhibition caused apoptosis and decreased colony numbers in HNSCC cell lines harboring *NOTCH1* loss-of-function mutations (*NOTCH1*MUT) and reduced tumor size. PDK1 levels dropped following PI3K/mTOR inhibition in *NOTCH1*MUT but not *NOTCH1*WT HNSCC, and targeting PDK1 sensitizes *NOTCH1*WT HNSCC to PI3K/mTOR pathway inhibitors.[50] PI3K inhibitors or the dual mTOR/PI3K inhibitors led to a significant delay in resistance development in several cancer types including leukemias,[51] although resistance mechanisms to existing mTOR inhibitors exist.[52]

Notch has been linked to multiple biological functions, including regulation of self-renewal capacity, cell-cycle exit (in part through upregulation of p21/CDKN1A expression), and cell survival. In the stratified epithelium, Notch has a central role in promoting terminal differentiation, negatively regulated by EGFR, which is mediated through both direct effects (e.g., on activation of suprabasal keratins) and indirect effects on the Wnt, hedgehog, and interferon response pathways. In mature epithelium, expression of p63 (p53 family member) is highest in basal epithelial cells, where it functions as an inhibitor of NOTCH1 expression, and becomes downregulated during terminal differentiation coincident with NOTCH1 upregulation.[53] NOTCH1 or NOTCH2

mutations are detected in 75% of cutaneous SCC:[54] 10–19% of NOTCH1 in HNSCC.[49]

Notch signaling controls Snail expression by two distinct but synergistic mechanisms, including direct transcriptional activation of Snail and an indirect mechanism operating via lysyl oxidase (LOX). Notch increases LOX expression by recruiting hypoxia-inducible factor 1α(HIF-1α) to LOX promoter, which stabilizes the Snail protein, resulting in upregulation of EMT and migration and invasion of cancer cells.[36] The authors have shown that PKD1 regulates acinar-to-ductal metaplasia (ADM) by activating the Notch pathway, which previously had been established as a driver of acinar cell re-programming. On one hand, active PKD1 downregulated the expression of known suppressors of Notch (e.g., Cbl, Sel1 l). On the other hand, active PKD1 also upregulated the expression of Notch target genes (e.g., Hes-1, Hey-1), molecules that are involved in Notch signaling (e.g., MAP2K7), stem-cell markers (e.g., CD44), as well as proteinases, including Adam10, Adam17, and MMP7, that mediate Notch activation by S2 cleavage. PKD1 has been shown to activate nuclear factor κ-B (NF-κB), and NFκB and Notch both cooperate in some signaling pathways (reviews[55,56]). A crosstalk between Notch and canonical NF-κB-signaling pathways is needed for the progression of pancreatic cancer and PKD1 is a key enzyme linking Kras to Notch and NF-κB.[22,57,58,59] However, pancreatic cancer possess high frequency in KRas mutations (more than 90%), whereas according to most of the authors, BCC (and SCC-cutaneous and oral) is with low near 10–12% mutations in Ras family with several authors giving higher percentages.[27,60] In addition, several genes within the MAPK and PI3K pathways were identified as cooperating mutations for *KrasG12D*-driven PDAC, leading to the activation of these pathways, making PI3K a promising candidate for drug therapy in pancreatic ductal adenocarcinoma (PDAC).[61,62] Although moderate levels of p-AKT activity are detected in human BCCs, pharmacologic PI3K inhibitors inhibit BCC proliferation *in vitro* and *in vivo*.[63]

In addition to providing the mechanistic insight at a cellular level, Ganju *et al.* also demonstrated a reverse correlation between PKD1 and metastasis-associated protein 1 (MTA1) expression in samples of human prostate, colon, and breast cancers, in which PKD1 expression decreased and MTA1 expression increased, with progressed tumor grade or stage. MTA1 is a novel substrate for PKD1, and show that PKD1-mediated phosphorylation of MTA1 triggers its polyubiquitination and proteasomal degradation. PKD1-mediated downregulation of MTA1 was accompanied by a significant suppression of prostate cancer progression and metastasis in physiologically relevant spontaneous tumor models. Accordingly, progression of human prostate tumors to increased invasiveness was also accompanied by decreased and increased levels of PKD1 and MTA1, respectively.[64] Moreover, the authors propose that this leads to the shuttling of PKD1/β-catenin complexes to E-cadherin, with the effect of stabilizing cell–cell contacts.[37]

**10. PKD1 is activated during oxidative stress** through a mechanism that requires nonreceptor tyrosine kinases (c-Abl and Src) and PKCδ (and probably not other PKCs; Figure 5). c-Abl-dependent PKD1 phosphorylation at $Tyr^{463}$ (in the PH domain) releases intramolecular autoinhibition, and Srcdependent PKD1 phosphorylation at $Tyr^{95}$ creates a docking site for the C2 domain of PKCδ; PKCδ then phosphorylates the PKD1 activation loop at $Ser^{738}/Ser^{742}$. A redox-dependent pathway involving Src and c-Abl also promotes PKD1–PH domain phosphorylation at $Tyr^{432}$ and $Tyr^{502}$

(Figure 5), but the significance of these modifications is uncertain, because they do not lead to gross changes in PKD1 activity. There is evidence that the reactive oxygen species-activated PKD1 enzyme is localized (although not necessarily restricted) to mitochondria and that it recruits a nuclear factor κB (NFκB) pathway that induces expression of antioxidant/antiapoptotic genes (such as manganese superoxide dismutase) and promotes cell survival. It is noteworthy that the canonical growth factor-dependent PKD1-signaling pathway does not activate NFκB or induce manganese superoxide dismutase (mnSOD), emphasizing that the signaling repertoire and cellular actions of PKD1 can be highly contextual.[7]

Similar mechanism of activation mediated by a Src family kinase cascade was observed by Bolag after UVB exposure and UVB-elicited oxidative stress.[9] Although, according to authors, UVB increases tyrosine phosphorylation of PKD by Src as well as western analysis using an antibody recognizing phosphotyrosine463 (tyrosine residue phosphorylated by Abl, not by Src-authors' remark). This result is also consistent with the ability of the tyr463phe PKD mutant to act in a dominant negative manner to exacerbate UVB's apoptotic effect (and prevent PKD's ability to promote survival).[65] A number of studies have shown that PKD1 opposes the apoptotic effects of oxidative stress (or UVB exposure) in a variety of cells and allow survival of UV-damaged cells. UV-induced keratinocytes with DNA mutations could continue to proliferate and form skin tumors.[19,2]

Oncogene-induced senescence (OIS) is an initial barrier to tumor development. Reactive oxygen species (ROS) is critical for oncogenic Ras OIS, but the downstream effectors to mediate ROS signaling are still relatively elusive. Senescent cells develop a senescence-associated secretory phenotype (SASP). However, the mechanisms underlying the regulation of the SASP are largely unknown. The authors identify protein kinase D1 (PKD1) as a downstream effector of ROS signaling to mediate Ras OIS and SASP. PKD1 is activated by oncogenic Ras expression and PKD1 promotes Ras OIS by mediating inflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) via modulation of NF-κB activity. They demonstrate that ROS-protein kinase Cδ (PKCδ)-PKD1 axis is essential for the establishment and maintenance of IL-6/IL8 induction. In addition, the ablation of PKD1 causes the bypass of Ras OIS, and promotes cell transformation and tumorigenesis. Together, these

findings uncover a previously unidentified role of ROS–PKCδ–PKD1 pathway in Ras OIS and SASP regulation in IMR90 human diploid fibroblasts.[66]

The Warburg effect in cancer cells is the product of two factors, a return of cells to glycolytic metabolism and increased production of mitochondrial reactive oxygen species (ROS), which is due to alterations in oxidative phosphorylation. In established tumors, increased levels of oxidative stress are often accompanied by upregulation of antioxidant systems. The upregulation of antioxidant systems keeps ROS at levels, where they are protumorigenic and promote cell survival and proliferation, but do not induce apoptosis or necrotic cell death.[22]

An increased oxidative stress leads to PKD1 localization to the mitochondria, where it is activated. ROS-activated PKD1 has been shown not only to initiate cytosolic signaling pathways, but also to redistribute to the nucleus. The signaling pathway that leads to the activation of PKD1 by oxidative stress seems unique, because it involves tyrosine phosphorylation of the molecule at several residues, which do not occur when PKD1 is activated by receptor-mediated signaling.[22]

Another pathway of how PKD1 promotes cell survival is by activating extracellular signal-regulated kinases 1/2 (ERK1/2), which confers a protective response to chronic oxidative stress, and by downregulating c-Jun *N*-terminal kinase (JNK) signaling that promotes apoptosis. Similarly, the downregulation of p38 MAPK signaling by PKD1 in response to hydrogen peroxide has been demonstrated to protect cells from apoptosis. Another target for ROS-activated PKD1 is the small heat-shock protein Hsp27, which is phosphorylated by PKD1 at $S^{82}$. PKD1-phosphorylated Hsp27 can bind apoptosis signal-regulating kinase 1 to prevent JNK-induced apoptosis. Hsp27 also has been implicated in chemoresistance of several cancers. In addition, the tumor suppressor death-associated protein kinase phosphorylates and activates PKD1 in response to oxidative damage. Such signaling induces autophagy, due to PKD1-mediated phosphorylation of Vps34, which increases its lipid kinase activity and autophagosome formation.[22,57]

PKD1 acts as a sensor for mitochondrial oxidative stress and regulates the cellular response by activating the NFκB pathway. PKD1 activates NFκB by the phosphorylation and activation of inhibitory κ kinase (IKK). This results in the degradation of inhibitory protein IκB and the release of

NFκB from the inhibitory complex, followed by accumulation in the nucleus and induction of downstream target genes, which in turn causes cell survival, cell proliferation, and inflammation. PKD1 may also be involved in IKK independent mechanisms that activate the NFκB pathway within

cells. PKD1-mediated NFκB activation has been shown in different cell lines, including immune cells, intestinal epithelial cells, and lung cells. However, only PKD1 activation following tyrosine phosphorylation is capable of activating the NFκB pathway, which may point to specific requirements, including specific structural changes in PKD1, for NFκB activation.[4] FκB pathway induces expression of antioxidant/antiapoptotic genes (such as manganese superoxide dismutase) and promotes cell survival. It is noteworthy that the canonical growth factor-dependent PKD1-signaling pathway does not activate NFκB or induce manganese superoxide dismutase, emphasizing that the signaling repertoire and cellular actions of PKD1 can be highly contextual.[7] More work is needed to understand and establish the underlying structural requirements and functional mechanism involved in PKD1-mediated NFκB activation.[4]

Downstream of PKD1, activation of NF-κB was linked to increased expression of *SOD2*, a gene-encoding manganese superoxide dismutase (MnSOD). MnSOD generates hydrogen peroxide, a bona fide-signaling molecule that is important for tumor-cell proliferation.[57]

Protein kinase D1 (PKD1) has been reported to initiate protective signaling against oxidative stress or ischemia, two conditions that impinge on the induction of ER stress. In addition, the high levels of expression of PKD1, observed in highly proliferative cancers and tumors with poor prognosis, contribute to enhanced resistance to chemotherapy. In recent study, Wu S *et al.* show that the ER stress inducers tunicamycin and thapsigargin lead to the activation of PKD1 in human prostate cancer PC-3 cells and in hepatoma HepG2 cells through a PKCδ-dependent mechanism. Moreover, their data indicate that PKD1 is required for the stabilization of inositol-requiring enzyme 1 (IRE1) and the subsequent regulation of its activity. PKD1 activation contributes to the phosphorylation of mitogen-activated protein kinase phosphatase 1, resulting in decreased IRE1-mediated c-Jun N-terminal kinase activation.[67]

In recent paper Chen J *et al.* results have shown that PKD1 not only mediates the growth and apoptosis of SCC25 cancer cells in the hypoxic environment, but also regulates the glycolytic metabolism of cancer cells (GLUT1 and LDHA expression) by promoting glucose uptake and expression of HIF-1α and glycolytic enzymes in a hypoxic environment. Inhibition of the expression and activation of PKD1 can significantly inhibit glycolytic metabolism of cancer calls as well as create an acidic tumor microenvironment. Tey found that PKD1 is associated with the activation of p38 MAPK signaling and the activation of p38 MAPK signaling is necessary for HIF-1α accumulation and nuclear translocation.[142]

**11. PKD1-mediated activation of NF-κB** also increases the expression of epidermal growth factor receptor (EGFR) and its ligand-transforming growth factor-alpha (TGFα) and epidermal growth factor (EGF). In the presence of an oncogenic KRas mutation, these lesions can then further develop to pancreatic cancer. KRas/mROS/PKD1/NF-κB signaling contributes to tumor initiation by upregulating expression of EGFR and the ligands mentioned above. EGFR signaling then elevates overall (oncogenic and wild-type) Kras activity to pathological levels to drive the formation of pancreatic precancerous lesions.[57,68]

Decreased expression of EGFR as a result of PKD1 knockdown was also observed from us in hTert keratinocytes.[69]

**12. PKD function in inflammation:** A recent study using pancreatic β-cells demonstrated that stress signals markedly induced TNFAIP3/A20, a zinc finger-containing, immediate early response gene with potent antiapoptotic and anti-inflammatory functions. In fact, A20 is an early NF-κB-responsive gene that encodes an ubiquitin-editing protein that is involved in the negative feedback regulation of NF-κB signaling. Interestingly, other studies demonstrated that PKD induces A20 promoter activity. It is plausible that PKD initiates not only an inflammatory response via NF-κB, but also stimulates the expression of the antiapoptotic and anti-inflammatory A20 as a feedback mechanism that protect cells subjected to stress signals, including oxidative stress (Figure 5).[2]

NF-κB is a key transcription factor that is activated by multiple receptors and regulates the expression of a wide variety of proteins that control innate and adaptive immunity. A number of studies indicate that PKD is a mediator of NF-κB induction in a variety of cells exposed to GPCR agonists except oxidative stress. In view of the increasing recognition of the interplay between inflammation and cancer development, a possible role of PKD in linking these processes is of importance. However, the precise molecular mechanisms remain incompletely understood.[2]

Stimulation of human colonic epithelial NCM460 cells with the GPCR agonist and bioactive lipid lysophosphatidic acid (LPA) led to a rapid and striking activation of PKD2, the major isoform of the PKD family expressed by these cells. LPA stimulated the production of interleukin 8 (IL-8), a potent proinflammatory chemokine, and stimulated NF-κB activation. PKD2 gene silencing dramatically reduced LPA-stimulated NF-κB promoter activity and IL-8 production. These results imply that PKD2 mediates LPAstimulated IL-8 secretion in NCM460 cells through an NF-κB-dependent pathway. PKD2 has also been implicated in mediating NF-κB activation by Bcr-Abl in myeloid leukemia cells. Prostaglandins (e.g., PGE2) produced through cyclooxygenase-2 (COX-2; PGHS-2—prostaglandin H synthase) play a critical role in colon cancer development, and colonic myofibroblasts are major contributors to their generation. Recent results demonstrated that the knockdown of PKD1 in these cells prevented the synergistic increase in COX-2 expression induced by the proinflammatory mediators bradykinin and tumor necrosis factor (TNF-α). Thus, these novel results raise the attractive possibility that PKD plays a critical role in mediating COX-2 expression in response to potent proinflammatory mediators in human colonic myofibroblasts.[2]

NF-κB also plays a critical role in inflammatory and cell death responses during acute pancreatitis. The PKC isoforms PKCδ and ε are key regulators of NF-κB activation induced by cholecystokinin-8 (CCK-8), an agonist that induces pancreatitis when administered to rodents at supramaximal doses.[2,59] The studies showed that PKD/PKD1, the major PKD isoform in rat pancreatic acinar cells, is activated by phorbolesters and multiple pancreatic secretagogues, including cholecystokinin-8 (CCK-8 or CCK), the cholinergic agonist carbachol (CCh), and bombesin, but not by growth factors. In isolated rat pancreatic acinar cells, CCK and CCh induced a dose-dependent rapid activation of PKD as measured by an in vitro kinase assay and by phosphorylation at the activation loop ($Ser^{744/748}$) or autophosphorylation site ($Ser^{916}$). The secretagogue-stimulated PKD activation and phosphorylation occurs predominantly through a PKC-dependent pathway without involvement of Src/PI3K/MAPK/tyrosine phosphorylation. Using isoform-specific inhibitory peptides for PKCε and δ and genetic deletion of PKCε and δ in pancreatic acinar cells, the authors further identified PKD as an early convergent point for PKCε and δ in the signaling pathways triggered by the pancreatic secretagogues.[59]

Acinar cells elaborate a plethora of digestive enzymes that are synthesized and stored as inactive zymogen precursors to avoid autodigestion. Various *in vitro* and *in vivo* studies have established that the intra-acinar activation of zymogen is a key early event in the pathogenesis of pancreatitis. Activation of trypsinogen and other zymogens has been observed in the pancreatic homogenate as early as 10 min after supramaximal stimulation by cerulein in rats and increases over time. The fact that other markers of pancreatitis, e.g., hyperamylasemia, pancreatic edema, and acinar cell vacuolization, can only be detected 30 min. after supramaximal stimulation strongly supports the paradigm that zymogen activation is the cause and not the result of pancreatitis. Evidence points to the pivotal role played by the novel isoforms of PKC, PKCδ, and PKCε, in zymogen activation. Following specific and selective inhibition of secretagogue-induced PKD activation and autophosphorylation at $Ser^{916}$ by CRT0066101 (PKD inhibitor), secretagogueinduced zymogen activation was markedly reduced without affecting basal zymogen activation or secretion. Another exciting result was that the stimulation of amylase secretion by a pathologic dose of secretagogue (either 100 nM CCK, 1 mM CCh, or 10 lM bombesin) was significantly reduced following CRT0066101 pretreatment. However, CRT0066101 pretreatment did not reduce amylase secretion induced by physiological concentrations of CCK (0.1 nM), CCh (1 lM), or bombesin (10 nM). Interestingly, similar results were also achieved by another research group using molecular approaches in mouse pancreatic acinar cells. The authors found that CCK-induced cathepsin-B activity was inhibited by CRT0066101, indicating that PKD regulates cathepsin-B-induced trypsinogen activation. Authors' studies suggest that PKD is a potential mediator of zymogen activation and amylase secretion in pancreatitis. These events, together with NF-κB activation, are all early events that contribute to the pathologic process of acute pancreatitis (Figure 6). Their results further indicate

that PKD is a potential target for therapy in the early stage of the disease.[59]

In contrast, PKD3 is the predominant isoform expressed in mouse pancreatic acinar cells, and is also activated by gastrointestinal hormone or cholinergic stimulation.[59]

*PRKD1* and its gene product serine–threonine kinase protein kinase D1 (PKD1) are not expressed in normal human pancreatic acinar cells, but contribute to insulin secretion in islets. PKD1 mRNA and protein expression is upregulated in acinar cells during early events that contribute to formation of pre-neoplastic lesions. Consequently, it remains elevated in human patient samples of PDA (pancreatic adenocarcinoma). However, there are no data available correlating PKD1 expression or activity with patient outcome. Overexpression or activation of PKD1 in PDA cell lines functionally was linked to increased proliferation and survival. *In vivo*, an acinar cell-specific knockout of PKD1 significantly decreased the formation and progression of KRas-induced precancerous lesions, and an inhibition of PKD decreased orthotopic growth of pancreatic tumor-cell lines in mice. Although increased expression of PKD1 correlates with the presence of oncogenic KRas in precancerous pancreatic lesions, only little is known of how the *PRKD1* promoter is regulated during cancer development. By discovery that KRas-mediated induction of canonical NF-κB signaling regulates the *PRKD1* promoter, Doppler and Storz described a novel mechanism that governs PKD1 expression in PDA (see below).[57] In addition, increased PKD1 activity can be detected in regions of pancreatitis, and expression in ADM, PanIN1, and PanIN2 pre-neoplastic pancreatic lesions[57] and in pancreatic cancer.[70,4,59]

Group B streptococci (GBS) are one of the leading causes of life-threatening illness in neonates. Proinflammatory responses to GBS mediated through host innate immune receptors play a critical role in the disease manifestation. In a study, Upadhyay *et al.* investigated the role of protein kinase D (PKD)1 in the proinflammatory responses to GBS. They found that both live and antibiotic-killed GBS induce the activation of PKD1 through a pathway that is dependent on the TLR-signaling adaptor MyD88 and its downstream kinase IL-1R-associated kinase 1, but independent of TNFR-associated factor 6. Their studies using pharmacological PKD inhibitors and PKD1-knockdown macrophages revealed that PKD1 is indispensable for GBS-mediated activation of MAPKs and NF-κB and subsequent expression of proinflammatory mediators. Furthermore, systemic administration of a PKD inhibitor protects d-galactosamine-sensitized mice from shock-mediated death caused by antibiotickilled GBS. These findings imply that PKD1 plays a critical regulatory role in GBS-induced proinflammatory reactions and sepsis, and inhibition of PKD1 activation together with antibiotic treatment in GBS-infected neonates could be an effective way to control GBS diseases.[71]

Protein kinase D (PKD) was recently implicated in mediating pro-inflammatory macrophage responses to GBS outside of the reproductive system. Recent work aimed to characterize the human placental macrophage inflammatory response to GBS and address the extent to which PKD mediates such effects. Primary human placental macrophages were infected with GBS in the presence or absence of a specific, small molecule PKD inhibitor, CRT 0066101. GBS evoked a strong inflammatory phenotype characterized by the release of inflammatory cytokines (TNFα, IL-1β, IL-6 ($P \leq 0.05$), NLRP3 inflammasome assembly ($P \leq 0.0005$), and NFκB activation ($P \leq 0.05$). Pharmacological inhibition of PKD suppressed these responses, newly implicating a role for PKD in mediating immune responses of primary human placental macrophages to GBS.[72]

The myofibroblast is a gastrointestinal stromal cell that is a target of tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine strongly implicated in colitis-associated cancer. Crosstalk between TNF-α and other pro-inflammatory mediators amplify inflammatory signaling but the mechanism is unknown. Angiogenin (ANG) is a 14-kDa angiogenesis protein that is regulated in patients with inflammatory bowel disease. TNF-α/BK enhance PKD phosphorylation and COX-2 expression in primary mouse and human colonic myofibroblasts. Angiogenin is produced by the myofibroblast, and inhibition of ANG signaling, either by its absence (ANG-KO) or by pharmacologic inhibition, blocks enhanced PKD phosphorylation and synergistic COX-2 expression induced by TNF-α/BK. ANG mediates crosstalk signaling between TNF-α/BK in the regulation of stroma-derived COX-2 and may be a novel therapeutic target for the management of colitis-associated cancer.[73]

**13. PKD function in the immune response.** A prominent PKC/PKD axis has been demonstrated in B and T lymphocytes. PKD is cytosolic in unstimulated T cells, but it rapidly polarizes to the immunological synapse in response to antigen/antigen presenting cells. PKD repositioning is driven by the accumulation of DAG at the immunological synapse. As in other cell types, PKD in lymphocytes phosphorylates and regulates class II HDACs. PKD family has also been implicated in regulating the activity of β1-integrins in T cells via Rap1 and IL-2 promoter in response to TCR (T-cell receptor) stimulation.[2] Endogenous PKD1 exhibits a constitutive association with Btk. The activation of PKD1 in BCR cross-linking depends on Syk and PLCγ activity and is also regulated by Btk. It was suggested that PKD1 could function in a feedback loop, negatively regulating the BCR by reducing the ability of Syk to phosphorylate PLCγ.[6]

PKD2 is the major PKD isoform expressed in lymphoid tissues, but PKD2 catalytic activity is not essential for the development of mature peripheral T- and B-lymphocytes. PKD2 catalytic activity is, however, required for efficient antigen receptor-induced cytokine production in T lymphocytes and for optimal T-cell-dependent antibody responses *in vivo*. Results reveal a key *in vivo* role for PKD2 in regulating the function of mature peripheral lymphocytes during adaptive immune responses. They also confirm the

functional importance of PKC-mediated serine phosphorylation of the PKD catalytic domain for PKD activation and downstream signaling and reveal that different PKD family members have unique and non-redundant roles *in vivo*.[74]

Recently, it was found that PKD3 is abundantly expressed in B cells, and as for PKD1, BCR engagement results in strong activation of PKD3. PLCγ and novel PKC isoforms are required in this activation process.[6]

Toll-like receptors (TLRs) have been identified as primary innate immune receptors. TLRs distinguish between different patterns of pathogens and activate a rapid innate immune response. Recent results implicated PKD in TLR 2, 5, and 9 functions in different cell types. Specifically, PKD is a downstream target in TLR9 signaling in macrophages and TLR2 in mouse bone-marrow-derived mast cells. Macrophages express all three PKD family members and bacterial DNA and class B type CpG DNA (CpG-B DNA) induce activation of PKD1. Upon CpG-B DNA stimulation, PKD1 interacted with the TLR9/MyD88/IL-1R-associated kinase/ TNFR-associated factor 6 complex. Knockdown of PKD1 revealed that PKD1 is required for the activation of NF-κB and MAPKs and subsequent expression of cytokines in response to CpG-B DNA.[75] The TLR-interacting protein myeloid differentiation factor 88 (MyD88) has been suggested to play a role in TLR-induced PKD activation.[2] The binding of MyD88 to TLR9 leads to the subsequent recruitment of interleukin-1 receptor-associated kinase (IRAK) family members, IRAK4 and IRAK1. While IRAK1 was critical for the initial phase, IRAK2 was required for the late phase of TLR9 signaling by sustaining activation of PKD1 that leads to the activation of NF-κB and MAPKs.[76]

PKD1 is recruited to the TLR9-receptor signaling complex and physically interacts with IRAK4, IRAK1, and TRAF6 upon CpG DNA stimulation. Activation of PKD1 by CpG DNA is dependent on IRAK4 and IRAK1, while it is independent of TRAF6. While IRAK1 was critical for the initial phase, IRAK2 (regulation of Irak-M expression) was required for the late phase of TLR9 signaling by sustaining activation of PKD1 that leads to activation of NF-κB and MAPKs (p38, ERK, JNK). IRAK-M expression induced by TLR ligand stimulation is one of the central negative feedback mechanisms for the TLR-initiated innate immune response. Studies with IRAK-M-deficient (Irak-m2/2) mice confirmed IRAK-M as a negative regulator of TLR/IL-1R signaling in monocytic cells.[76] Although the precise role of PKD in TLR function remains incompletely understood, these studies provide evidence, indicating that PKD plays a role in the regulation of the innate immune response mediated by this class of pattern recognition receptors.[2]

There have been a couple of studies that indicate a possibility that PKD family members may be involved in TLR signaling in nonimmune cells. LPS-mediated p38 activation and TNF-α secretion in microglial cells is suppressed by a PKC/PKD inhibitor, Go6976. PKD1 has been shown to bind and phosphorylate TLR5, and Go6976 suppresses flagellin mediated p38 activation and IL-8 production in epithelial cells.[75]

In addition, PKD-mediated phosphorylation of TLR5 on $Ser^{805}$ appears necessary for TLR5 response to its ligand, flagellin (bacterial protein). TLR5 phosphorylation contributes to p38MAPK activation and production of inflammatory cytokines in epithelial cells.[2] Flagellin stimulation of cells activated PKD1, and inhibition of PKD1 reduced flagellin-induced interleukin-8 (IL-8) production in epithelial cells. Steiner *et al.* found that the inhibition of either kinase with shRNA reduced IL-8 and CCL20 release due to TLR4 and TLR2 agonists to a similar extent as previously reported for TLR5. PKD1 and PKD2 inhibition reduced NF-κB activity but not MAPK activation. These results demonstrate that both PKD1 and PKD2 are required for inflammatory responses following TLR2, and TLR5 activation, although PKD1 is more strongly involved. These kinases likely act downstream of the TLRs themselves to facilitate NF-κB activation but not MAP kinase phosphorylation.[33]

Many catabolic stimuli, including interleukin-1 (IL-1) in combination with oncostatin M (OSM), promote cartilage breakdown via the induction of collagen-degrading collagenases such as matrix metalloproteinase 1 (MMP1) and MMP13 in human articular chondrocytes. Indeed, joint diseases with an inflammatory component are characterised by excessive extracellular matrix (ECM) catabolism. Importantly, protein kinase C (PKC) signalling has a primary role in cytokine-induced MMP1/13 expression, and is known to regulate cellular functions associated with pathologies involving ECM remodelling. Substrates downstream of PKC remain undefined. Herein, Baker J *et al.* show that both IL-1- and OSM-induced phosphorylation of protein kinase D (PKD) in human chondrocytes is strongly associated with signalling via the atypical PKCι isoform. Consequently, inhibiting PKD activation with a pan-PKD inhibitor significantly reduced the expression of MMP1/13. Specific gene silencing of the PKD isoforms revealed that only PKD3 (PRKD3) depletion mirrored the observed MMP repression, indicative of the pharmacological inhibitor specifically affecting only this isoform. PRKD3 silencing was also shown to reduce serine phosphorylation of signal transducer and activator of transcription 3 (STAT3) as well as phosphorylation of all three mitogen-activated protein kinase groups. This altered signalling following PRKD3 silencing led to a significant reduction in the expression of the activator protein-1 (AP-1) genes FOS and JUN, critical for the induction of many MMPs including MMP1/13. Furthermore, the AP-1 factor activating transcription factor 3 (ATF3) was also reduced concomitant with the observed reduction in MMP13 expression. Taken together, the authors highlight an important role for PKD3 in the pro-inflammatory signalling that promotes cartilage destruction.[77]

**14. PKD in fibtoblasts -** Recently, CID755673 (2,3,4,5-Tetrahydro-7-hydroxy-1H-benzofuro[2,3-

c]azepin- 1-one) has been reported to act as a highly selective inhibitor of the catalytic activity of members of the PKD family. In Swiss 3T3 fibroblasts, a cell line used extensively as a model system to elucidate mechanisms of GPCR signaling, PKD1 expression potently enhances mitogenic responses induced by Gq-coupled receptor agonists, including increased DNA synthesis. In the course of experiments using CID755673 in this system, Torres-Marquez *et al.* found that this compound exerted unexpected and potent stimulatory effects on [3H]thymidine incorporation and cell cycle progression in cells stimulated with bombesin, a G protein-coupled receptor agonist, phorbol 12,13-dibutyrate (PDBu) or epidermal growth factor (EGF). These stimulatory effects could be dissociated from the inhibitory effect of CID755673 on PKD activity, since enhancement of DNA synthesis was still evident (even increased – our comment) in cells with severely down-regulated PKD1 after transfection of siRNA targeting PKD1. The authors' results imply that CID755673 has other cellular target(s) in addition to PKD and therefore, experiments using this compound to elucidate the role of the PKD family in cell regulation should be interpreted with caution. Thus, at least CID755673 enhances mitogenic signaling by phorbol esters, bombesin and EGF through a protein kinase D-independent pathway and even, probably inhibit fibroblast proliferfation, the effect opposing that in human keratinocytes.[237]

Su Y *et al.* report that the classical protein secretory pathway components, including PKD1, DAG, PLD1, ARF1 and PI4KIIIβ, play a vital role in modulating secretion of senescence-associated secretory phenotype (SASP) factors and their function. Perturbation of this pathway affects the establishment of Ras oncogene-induced senescence (OIS), and can induce death in senescent fibroblasts and cancer cells. [238]

They observed that impairment or deprivation of the essential components of classical protein secretory pathway, such as PKD1, DAG, PLD1, ARF1 and PI4KIIIβ, decreased IL-6/IL-8 secretory levels during Ras OIS and eventually retarded Ras OIS. These results imply that the PKD1-mediated classical protein secretory pathway is not merely involved in regulating protein secretion, but also involved in Ras OIS responses through mediating IL-6/IL-8 secretion and the establishment of the IL-6/IL-8 autocrine network. Previously, the authors identified PKD1 acted as a downstream effector of the reactive oxygen species (ROS)-protein kinase Cδ (PKCδ) cascade to mediate Ras OIS by regulating IL-6/IL-8 expression via modulation of NF-κB activity. Therefore, they propose that, during Ras OIS, the ROS–PKCδ–PKD1 axis modulates SASP factor expression, and the PKD1-mediated classical protein secretory pathway from the TGN to the cell surface regulates SASP factor secretion. Moreover, both processes are not exclusive and they all have significant impact on the Ras OIS response. In summary, the authors show that the PKD1-mediated classical protein secretory pathway is activated in senescent cells and is essential for SASP factor secretion. Ablation of this pathway either delays the onset of Ras OIS, or triggers senescent cell death. [238]

Senescent cells accumulate in various tissues and organs with age. Selective elimination of senescent cells prevent or delay age-related disorders and extend healthspan in mouse model. In addition, cancer therapy-induced senescence (TIS) can induce senescence and SASP in tumor cells. Although TIS facilitates cancer treatment outcome because of its associated immune surveillance, the resident and viable senescent cells *in vivo* may cause cancer relapse later. Therefore, selective removal of normal senescent cells may improve healthy aging, and selective eradication of TIS cells may promote the long-term efficacy of TIS. However, selective clearance of senescent cells is a challenging task. SASP-competent TIS cells exhibit hypermetabolic activity and autophagic activity, which present as a senescent cell-specific vulnerability that can be selectively targeted to induce senescent cell death.[238]

Myofibroblasts are gastrointestinal tract stromal cells that regulate the reparative process and are known targets of inflammatory mediators including bradykinin (BK). However, the mechanisms through which inflammation regulates myofibroblast-induced wound healing remain incompletely understood. Here, Chu E *et al.*, demonstrate, for the first time, that BK stimulates myofibroblast migration through protein kinase D (PKD)-mediated activation of the cyclooxygenase-2 (COX-2) and heat shock protein 27 (Hsp27) pathways.[239]

S1P receptors couple to $G\alpha_{12/13}$ and activate PKD through a pathway that involves the synergistic actions of PKC and RhoA (a member of the Rho family of small GTPases) in some cell types. While Thr also signals through the $G\alpha_{12/13}$-RhoA pathway in many cell types, this mechanism for Thr-dependent PKD activation has never been reported and the notion that the RhoA-dependent pathway might specifically activate only certain isoforms of PKD has never been considered. Therefore, PKD isoform activation was examined in cardiac fibroblasts pretreated with the *C. botulinum* C3 toxin, which specifically ADP-ribosylates and functionally inactivates Rho proteins. C3 toxin pretreatment completely abrogates S1P- and Thr-dependent activation of PKD2 and PKD3, as evidence by a complete block of the agonist-dependent PKD2 and PKD3 band shifts and increases in $Ser^{744}/Ser^{748}$ and $Ser^{916}$ phosphorylation. Of note, PKD isoform activation by PDGF also is attenuated by C3 toxin, whereas PKD isoform activation by $H_2O_2$ or PMA is largely preserved in C3 toxin-treated cultures. These results identify a specific role for Rho in the PKD2 and PKD3 activation pathway triggered by $G\alpha_{12/13}$-coupled S1P and Thr receptors and show that Rho is not required for PKD2 and PKD3 activation during oxidative stress in cardiac fibroblasts.[240]

**15. PKD in endothelial cells** - PKDs regulate both hypoxia-induced VEGF expression/secretion by the tumor cells and VEGF- stimulated angiogenesis, which are essential for the malignant progression of tumors.

More and more studies show that PKD-1 signaling regulates the transcriptional expression of genes that are important in angiogenesis. Recently published review provided the insights into molecular mechanisms by which PKD1 regulates transcriptional expression of angiogenicgenes, focusing on the transcriptional regulation of CD36 by PKD-1- FoxO1 signaling axis along with the potential implications of this axis in arterial differentiation and morphogenesis. Ren discussed a new concept of dynamic balance between proangiogenic and antiangiogenic signaling in determining angiogenic switch, and stress how PKD-1 signaling regulates VEGF signaling-mediated angiogenesis. Histone acetylation/deacetylation regulates transcriptional expression of genes through a dynamic balance between histone acetyltransferases and histone deacetylases (HDACs). HDACs are critical to inhibiting acetylation of nucleosome histones. Interestingly, HDAC5 and HDAC7, highly expressed in ECs ((vascular) endothelial cells), are the substrate of PKD-1 and regulate EC functions and angiogenesis.VEGF stimulates HDAC5 phosphorylation and nuclear export in ECs *via* a VEGF Receptor 2 (VEGFR2)-PLCγ1-PKCα-PKD- dependent pathway. Moreover, PKD-1signaling interacts with HDAC5 to promote transcriptional activation of myocyte enhancer factor-2 (MEF2) and a specific subset of gene expression in response to VEGF including NR4A1, an orphan nuclear receptor involved in angiogenesis. VEGF-mediated PKD-1 signaling also stimulates HDAC7 phosphorylation and cytoplasmic accumulation, thus modulating the expression of HDAC7-targeting and VEGF-response genes as well as VEGF-induced angiogenic gene expression, including matrix metalloproteinases MT1-matrix metalloproteinase (MT1-MMP (MMP-14)) and MMP10 expression, VEGF- stimulated EC migration, tube formation, and sprouting angiogenesis. A similar mechanism is involved in the induction of PDGF-B/PDGFR-P expression and subsequent proangiogenic responses. PKD-1 interacts with specific HDACs to function as a molecular switch for controlling angiogenic gene transcription and VEGF-mediated angiogenesis.[15,78]

Ren found that PKD-1 signaling in microvascular ECs (MVECs) promotes nuclear accumulation of HDAC7 in response to lysophosphatidic acid (LPA), which is different from a previous report in human umbilical vein endothelial cells (HUVECs) exposed to VEGF. This indicates that a PKD- 1 signaling "signature" is different in a specific cellular context. In certain cellular microenvironments, PKD-1 determines the formation of nuclear regulatory complex and aids in gene locusspecific nucleosomal enrichment of specific histone deacetylase. PKD-1 may mediate HDAC7-FoxO1 interaction in the nucleus and maintain context relevant EC functions *via* FoxO1-dependent regulation of CD36 transcription in HMVECs rather than *via* MEF-2- dependent regulation of matrix metalloprotease.[15]

Recent paper reported Vascular endothelial growth factor A (VEGF) signals primarily through its cognate receptor VEGF receptor-2 (VEGFR-2) to control vasculogenesis and angiogenesis, key physiological processes in cardiovascular disease and cancer. In human umbilical vein endothelial cells (HUVECs), knockdown of protein kinase D-1 (PKD1) or PKD2 down-regulates VEGFR-2 expression and inhibits VEGF-induced cell proliferation and migration. However, how PKD regulates VEGF signaling is unclear. Previous bioinformatics analyses have identified binding sites for the transcription factor activating enhancer-binding protein 2 (AP2) in the *VEGFR-2* promoter. Using ChIP analyses, the authors found that PKD knockdown in HUVECs increases binding of AP2β to the *VEGFR-2* promoter. Luciferase reporter assays with serial deletions of AP2-binding sites within the *VEGFR-2* promoter revealed that its transcriptional activity negatively correlates with the number of these sites. Next they demonstrated that AP2β up-regulation decreases VEGFR-2 expression and that loss of AP2β enhances VEGFR-2 expression in HUVECs. *In vivo* experiments confirmed increased VEGFR-2 immunostaining in the spinal cord of AP2β knockout mouse embryos. Mechanistically, they observed that PKD phosphorylates **AP2β** at $Ser^{258}$ and $Ser^{277}$ and suppresses its nuclear accumulation. Inhibition of PKD activity with a pan-PKD inhibitor increased AP2β nuclear localization, and overexpression of both WT and constitutively active PKD1 or PKD2 reduced AP2β nuclear localization through a $Ser^{258}$- and $Ser^{277}$-dependent mechanism. Furthermore, substitution of $Ser^{277}$ in AP2β increased its binding to the *VEGFR-2* promoter.[80,79]

One of the effector kinases that become activated in endothelial cells upon VEGF treatment is protein kinase D (PKD). Aicart-Ramos C *et al.* showed that PKD phosphorylates eNOS on Ser1179 afteer VEGF or phorbol 12,13-dibutyrate (PDBu) treatment, leading to its activation and a concomitant increase in NO synthesis and consequent vasodilation. In addition, pharmacological inhibition of PKD and gene silencing of both PKD1 and PKD2 abrogate VEGF signaling, resulting in a clear diminished migration of endothelial cells in a wound healing assay. Finally, inhibition of PKD in mice results in an almost complete disappearance of the VEGF-induced vasodilatation, as monitored through determination of the diameter of the carotid artery. Hence, their data indicate that PKD is a new regulatory kinase of eNOS in endothelial cells whose activity orchestrates mammalian vascular tone[241] and vasodilatation connected with matastasis.

**16. PKD is highly expressed in hematopoietic cells,** undergoes rapid and sustained activation upon stimulation of immune receptors, and is rapidly activated through a phosphorylation-dependent mechanism. A variety of stimuli, including phorbol esters and G-protein-coupled receptor and tyrosine kinase-linked receptor agonists, all lead to sustained PKD activation.[3]

Three platelet agonists were tested for their ability to activate PKD. Convulxin selectively activates the tyrosine kinase-coupled collagen receptor glycoprotein VI (GPVI), and thrombin activates protease-activated

receptor (PAR) receptors coupled to heterotrimeric $G_q$ and $G_{12/13}$ proteins. The thromboxaneA2 (TxA2) mimetic, U46619, activates platelets through the $G_q$- and $G_{12/13}$-coupled thromboxane prostanoid TP receptor. All the three of these agonists lead to PKC activation via PLC-mediated generation of DAG. Convulxin and thrombin stimulated a rapid four to fivefold increase in PKD catalytic activity as assessed by an in vitro kinase assay. PKD activity reached a maximum after 1 min of activation by either agonist and was maintained for 10 min. Similarly, Western blot analysis using the pS916 antiserum showed phosphorylation of PKD after 10 s, which was sustained for at least 10 min for convulxin and thrombin. These results suggest PKD activation as an early event in convulxin and thrombin signaling during platelet activation. PKD was also activated by U46619, but, in contrast to convulxin and thrombin, activation was shown to be weak and transient.[3]

Preincubating platelets with Ro31-8220 (a potent inhibitor of PKC, but not PKD) blocked PKD activation induced by PMA, convulxin, and thrombin. The results show that receptor activation of PKD is regulated by a PKC-dependent - signaling pathway in platelets, and that in spite of each agonist's having a different mechanism of activation, PKD is a common downstream signaling target during platelet activation.[3]

The magnitude and duration of PKD activation by maximal concentrations of PMA, convulxin, and thrombin are more or less identical. In addition, all three-stimulate PKD activation via PKC. This shows PKD as a common point of signal amplification downstream of tyrosine kinase- and $G_q$-coupled receptors. Convulxin-induced PKD activation was partially dependent on PI3 kinase and entirely dependent on the Src kinases. Because the activation of PLC$\gamma_2$ downstream of GPVI has similarly been shown to be dependent on these activities, this presumably reflects the degree of PKC activation in the presence of these inhibitors. Thrombin-induced PKD activation was also attenuated by the presence of the structurally different PI3-kinase inhibitors — LY294002 and wortmannin after 2 min of activation. This is consistent with the previous reports of a role for PI3-kinase in later stages of regulation of PKC by thrombin as shown by measurements of phosphorylation of pleckstrin.[3]

PKD activation is transient in response to weak platelet agonists U46619 and ADP (data not shown). This is likely to be the result of these agonists' activating PKC only temporarily, as sustained PKC activity is required to maintain PKD activation. After treatment with convulxin, thrombin, and PMA, a subsequent addition of Ro31-8220 led to a loss of PKD activity, suggesting that a phosphatase is active in platelets that can reverse PKD activation unless the PKC stimulus remains. Transient PKC activation was confirmed by measurements of phosphorylation of pleckstrin, an alternative PKC substrate in platelets. This transient regulation of PKD activation in response to weak agonists is unusual, as in other systems, PKD activation is sustained.[3]

The previous studies have implied that PI3-kinase is an important component of the synergy between $G_{i/z}$ and $G_q$ in the regulation of platelet aggregation, and the cited study also be the case for synergy at the level of PKC/PKD activation. A number of pathways that have been previously ascribed to PKC could in fact be regulated via PKD, because the activation of PKC always leads to PKD activation. This could potentially include all of the roles involving PKC, where the functional mechanisms remain ill-defined, such as aggregation and adhesion. Presently, however, there are no tools, such as selective inhibitors or mutant mice, to enable determination of the role of PKD function compared with the role of PKC in platelets. Future studies need to be performed to investigate whether PKC or PKD is responsible for a number of the effects downstream of PLC activation in platelets.[3]

**17. PKD – bone formation:** More specific to the bone microenvironment, PKD is stimulated by bone morphogenetic protein (BMP)-2 and IGF-I in mouse osteoblastic MC3T3 cells. In human bone marrow progenitor cells, activation of the PKD signaling cascade resulted in increased osterix production, a master osteoblastic transcription factor. Furthermore, studies in fetal rat calvarial cells have revealed the importance of PKD in the synergistic induction via BMP-7 and IGF-I of osteoblast differentiation and mineralized nodule formation. In addition, Yeh et al. used pharmacological inhibitors and transfection with constitutively active and catalytically inactive PKD constructs to effectively blunt PKD phosphorylation and osteoblast differentiation in response to BMP-7 and IGF-I stimulation, which plays a major role in bone formation. Together these studies have implicated PKD signaling in osteoblast function as a mediator of in vitro hormonal signaling at the cellular level. [243]

Dual-energy x-ray absorptiometry scan analysis of male and female pubescent mice demonstrated significantly decreased bone mineral density in the whole body and femoral bone compartments of PKD1 (+/−) mice, compared with their wild-type littermates. The body weight, nasal-anal length, and percentage body fat of the mice were not significantly different from their wild-type littermates. Cultured bone marrow stromal cells from PKD1 (+/−) mice demonstrated lower alkaline phosphatase activity in early differentiating osteoblasts and decreased mineralized nodule formation in mature osteoblasts. Quantitative RT-PCR analysis of osteoblast differentiation markers and osteoclast markers exhibited lower levels of expression in PKD1 (+/−) male mice than wild type. In female mice, however, only markers of osteoblast differentiation were reduced. PKD1 (+/−) mice also demonstrated a profound reduction in mRNA expression levels of BMP type II receptor and IGF-I receptor and in BMP-7 responsiveness in vitro. Together these data suggest that in mice, PKD1 action contributes to the regulation of osteoblastogenesis by altering gene expression with gender-specific effects on osteoclastogenesis, subsequently affecting skeletal matrix acquisition during puberty. [243]

Balanced osteoclast and osteoblast activity is necessary for skeletal health, whereas unbalanced osteoclast activity causes bone loss in many skeletal conditions. A better understanding

of pathways that regulate osteoclast differentiation and activity is necessary for the development

of new therapies to better manage bone resorption. The roles of Protein Kinase D (PKD) family

of serine/threonine kinases in osteoclasts have not been well characterized. In this study we use

immunofluorescence analysis to reveal that PKD2 and PKD3, the isoforms expressed in osteoclasts,

are found in the nucleus and cytoplasm, the mitotic spindle and midbody, and in association with

the actin belt. Leightner AC *et al.* show that PKD inhibitors CRT0066101 and CID755673 inhibit several distinct aspects of osteoclast formation. Treating bone marrow macrophages with lower doses of the PKD inhibitors had little effect on M-CSF + RANKL-dependent induction into committed osteoclast precursors, but inhibited their motility and subsequent differentiation into multinucleated mature osteoclasts, whereas higher doses of the PKD inhibitors induced apoptosis of the preosteoclasts. Treating post-fusion multinucleated osteoclasts with the inhibitors disrupted the osteoclast actin belts and impaired their resorptive activity. In conclusion, these data implicate PKD kinases as positive regulators of osteoclasts, which are essential for multiple distinct processes throughout their formation and function.[244]

Although PKD is broadly expressed and involved in numerous cellular processes, its function in osteoclasts has not been previously reported. In a study, Mansky KC *et al.* found that PKD2 is the main PKD isoform expressed in osteoclastic cells. PKD phosphorylation, indicative of the activated state, increased after 2–3 days of treatment of bone marrow macrophages with M-CSF and RANKL, corresponding to the onset of preosteoclast fusion. RNAi against PKD2 and treatment with the PKD inhibitor CID755673 showed that PKD activity is dispensable for induction of bone marrow macrophages into tartrate-resistant acid phosphatase-positive preosteoclasts in culture but is required for the transition from mononucleated preosteoclasts to multinucleated osteoclasts. Loss of PKD activity reduced expression of DC-STAMP in RANKL-stimulated cultures. Overexpression of DC-STAMP was sufficient to rescue treatment with CID755673 and restore fusion into multinucleated osteoclasts. From these data, the authors conclude that PKD activity promotes differentiation of osteoclast progenitors through increased expression of DC-STAMP.[245]

**18. PKD1 is also cleaved by caspase-3;** it is a component of the signaling machinery mobilized by proapoptotic stimuli (Figure 5c). Although there is general consensus that caspase-3 cleaves PKD1 at a site in the C1–PH interdomain, the precise cleavage site remains uncertain. The consequences of this proteolytic event (which removes the C1 domain, but not the "autoinhibitory" PH domain) also have been disputed. Vantus *et al.* (2004) concluded that PKD1 is a proteolytically activated enzyme on the basis of evidence that the PKD1 cleavage product generated during apoptosis displays a modest increase in basal activity compared with WTPKD1. However, Haussermann *et al.* (1999) showed that the C-terminal cleavage product (which lacks a C1 domain) does not respond to lipid cofactors (phosphatidylserine/PMA); as a result, the maximal activity of this catalytic fragment is inconsequential compared with the activity of the phosphatidylserine/ PMA activated full-length PKD1 enzyme. The notion that cleavage limits maximal PKD1 activity also is more consistent with recent results in cardiomyocytes, where the action of PKD1 to regulate lipoprotein lipasemediated triglyceride accumulation is lost during apoptosis (under conditions associated with the activation of caspase-3 and caspase-3-dependent cleavage of PKD1) (Figure 5).[7]

In addition, while inhibition/knockdown of PDK1 caused PKCη downregulation via the proteasomal pathway, the downregulation of PKCη caused by the depletion of PKCε or by PKC inhibitors was independent of the proteasome mediated pathway. Another study reported that the de-phosphorylation of PKCη was mediated by integrin-associated serine–threonine phosphatase PP1γ in human platelets which was shown to be independent of the ubiquitin-mediated degradation. In addition, differential expression analysis in the neoplastic cell line 8701-BC demonstrated that PKCη downregulation can be induced by type V collagen.[81] These results have shown not only different pathways for PKCη regulation, but also that PKD1 is a kinase which participate in this process (Figure 7).

In addition, senescent cells develop senescence-associated secretory phenotype (SASP) which possesses multiple biological functions via autocrine or paracrine manner. However, the status of the protein kinase D1 (PKD1)-mediated classical protein secretory pathway from *trans*-Golgi network (TGN) to cell surface during cellular senescence and its role in cellular senescent response remain unknown. The activities or quantities of the critical components of this pathway including PKD1, DAG, PLD1, ARF1, and PI4KIIIβ at TGN are increased in senescent cells (fibroblasts). Block of this pathway decreases IL-6/IL-8 secretion and results in IL-6/IL-8 accumulation in SASP-competent senescent cells. Intervention of this pathway reduces IL-6/IL-8 secretion during Ras OIS, retards Ras OIS and alleviates its associated ER stress and autophagy. Finally, targeting of this pathway triggers SASP-producing senescent cells death due to the intracellular accumulation of massive IL-6/IL-8. Together, authors' results unveil the hyperactivity state of protein secretory pathway in SASP-competent senescent cells and its critical functions in mediating SASP factors secretion and Ras OIS process, as well as in determining senescent cells fate.[8,79]

The involvement of PKDs in mediating such a diverse array of normal and abnormal biological activities in different subcellular compartments is likely to depend on the dynamic changes in their spatial and

temporal localization combined with their distinct substrate specificity.[5]

The phosphorylation reactions and docking mechanisms that influence the pharmacologic profile or signaling specificity of PKD1 present both challenges and opportunities for the development of novel PKD1-targeted pharmaceuticals for the treatment of cardiac hypertrophy/failure and certain intractable cancers.[7] In this context, it is important to point out that global knockout of PKD1 induces embryonic lethality in mice, with incomplete penetrance. Mice deficient in PKD1 enzymatic activity, via homozygous expression of PKD1 (S744A/S748A) "knock-in" alleles, also induced embryonic lethality.[2]

**Protein kinase D1, D2, and D3 in normal human keratinocytes**

Normal human epidermis demonstrated predominant PKD protein expression in the stratum basalis, the proliferative epidermal compartment, with decreased relative expression throughout the suprabasal strata.[11] Our results also showed the expression of PKD1 in proliferating subconfluent normal human keratinocytes, which resembles basal keratinocytes, although in very low mRNA (estimated only with Quantitative Real-Time PCR) and protein levels (detected only the active form p-PKD1 $^{Ser744/748}$ (Ser$^{738/742}$ in human—Figure 3) after short-term PMA stimulation).[10] The estimated function of the kinase in human keratinocytes is proproliferative and antidifferentiative, since knockdown of PKD1 using siRNA showed decrease mRNA expression of proliferative marker PCNA and increased expression of keratinocytes differentiative markers—K10 and Involucrin.[10] Uninvolved psoriatic skin showed a similar pattern of predominant PKD1 protein expression in the stratum basalis, but in contrast, involved (lesional) psoriatic epidermis demonstrated a diffuse pattern of staining with ectopic foci of increased staining intensity in the suprabasilar layers was observed. This result is consistent with altered PKD protein expression in the epidermis potentially mediating the antidifferentiative phenotype characterizing psoriasis.[11] According to Ryvkin *et al.* PKD1 is undetectable in human keratinocytes (KCs) and there is a divergence in the expression and function of other PKD isoforms, although the authors have used the same antibody for detection of PKD1 as Ristich *et al.*[11] and Ivanova *et al.*[69,10] — sc-935 (*Santa Cruz Biotech Inc*). Treatment of cultured human KCs with pharmacological inhibitors of PKDs resulted in growth arrest. The authors found that PKD2 and PKD3 are expressed differentially in proliferating and differentiating human KCs, with the former uniformly present in both compartments, whereas the latter is predominantly expressed in the proliferating compartment. Knockdown of individual PKD isoforms in human KCs revealed contrasting growth regulatory roles for PKD2 and PKD3. Loss of PKD2 enhanced KC-proliferative potential, while loss of PKD3 resulted in a progressive proliferation defect, loss of clonogenicity and diminished tissue regenerative ability. This proliferation defect was correlated with upregulation of CDK4/6 inhibitor p15$^{INK4B}$ and induction of a p53-independent G1 cell-cycle arrest. Simultaneous silencing of PKD isoforms resulted in a more pronounced proliferation defect consistent with a predominant role for PKD3 in proliferating KCs. These data underline the importance and complexity of PKD signaling in human epidermis and suggest a central role for PKD3 signaling in maintaining human epidermal homeostasis according to authors.[82]

Ryvkin *et al.* findings indicated that PKD3 silencing in NHKCs coincide with a marked reduction in p63, a major regulator of proliferation and differentiation in epidermis. Similar to p63, PKD3 is predominantly expressed in the proliferative compartment of epidermis and is downregulated in differentiated KCs. In addition, knockdown of either PKD3 or p63 leads to G1 cell-cycle arrest. However, p21 has been identified as a major mediator of G1 arrest induced by p63 silencing, whereas p21 transcript or protein levelsare not altered in PKD3 knockdown NHKCs. Ryvkin *et al.* have identified p15$^{INK4B}$ as a potential mediator of growth arrest induced by PKD3 silencing. p15$^{INK4B}$ is known to inhibit cyclin D-CDK4/6 complex formation and Rb phosphorylation, thus resulting in G1 cell-cycle arrest. p15$^{INK4B}$ is expressed at very low levels in proliferating KC, is upregulated upon differentiation and is a target of TGFβ-induced growth arrest. More recent studies have shown upregulation of both p15$^{INK4B}$ and p21 in response to the knockdown of p63 or Myc in human KCs suggesting a critical role for p15$^{INK4B}$ in regulating KC growth arrest. Therefore, mitogenic signaling transduced by PKD3 is likely to be critical in suppressing p15$^{INK4B}$ expression, hence maintaining KCs in the proliferative compartment of epidermis. In addition, dramatic reduction in p63 levels that follows PKD3 silencing suggests a role of PKD3 signaling in p63 stabilization. Because of key roles of p63 in cell growth and proliferation, apoptosis and differentiation, p63 levels are tightly regulated, mainly by the ubiquitin-dependent proteasomal degradation pathway. Recent studies have shown that degradation of p63 during KC differentiation by E3 ubiquitin ligase requires GSK3 kinase activity. Given that GSK3 is a potential substrate for PKD signaling, according to authors it is tempting to speculate that PKD3 signaling may stabilize p63 in proliferative KCs through negative regulation of GSK3.[82]

Epidermal tissue regenerated from PKD2-deficient KCs (sh-PKD2) showed a significant increase in epidermal thickness, although all differentiated layers of epidermis including granular and cornified layers were present. Immunostaining of tissue sections verified normal distribution of markers of early (INV) and late (FIL) epidermal differentiation; however, p63 staining was extended to upper spinous layers. On contrary, epidermal tissues regenerated from PKD3-deficient NHKCs were hypoplastic showing a marked differentiation defect as indicated by a lack of distinct spinous and granular layers and retention of nuclei in cornified layers (parakeratosis). Immunostaining revealed a marked reduction in p63 staining consistent

with the loss of KC-proliferative potential. Moreover, INV and FIL were expressed at markedly reduced levels indicating a differentiation defect.[82]

Additionally, ΔNp63α, implicated as an oncogene, is upregulated by activated Akt, part of a well-known cell survival pathway. Inhibition of Akt activation by phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and the presence of putative p63-binding sites in the PTEN promoter led the authors to investigate whether ΔNp63α regulates PTEN expression. Knockdown of ΔNp63α led to increases in PTEN levels and loss of activated Akt, while overexpression of ΔNp63α decreased PTEN levels and elevated active Akt. The repression of PTEN by ΔNp63α occurs independently of p53 status, as loss of ΔNp63α increases PTEN expression in cell lines with and without functional p53. In addition, decreased levels of ΔNp63α resulted in an increase in nuclear PTEN. Conversely, *in vivo* nuclear PTEN was absent in the proliferative basal layer of the epidermis where ΔNp63α expression is highest. Additionally, the authors showed that in keratinocytes a balance between ΔNp63α and PTEN regulates Akt activation and maintains normal proliferation rates. This balance is disrupted in non-melanoma skin cancers through increased ΔNp63a levels, and could enhance proliferation and subsequent neoplastic development.[83] Akt/PKB a serine/threonine kinase, is an important regulator of cell proliferation and survival. Akt alters cell cycle checkpoint control by either phosphorylating and inactivating $p21^{Cip/WAF1}$ or indirectly regulating the transcription of cyclin D1 (in keratinocytes not ERK1/2[84]) and $p27^{Kip1}$.[85]

In addition, in mouse keratinocytes a critical role for PKD1 during reversal of keratinocyte differentiation in culture, suggesting a potential proproliferative role in epidermal adaptive responses. Rashel *et al.* generated mice with targeted deletion of PKD1 in the epidermis to evaluate the significance of PKD1 in normal and hyperplastic conditions. These mice displayed a normal skin phenotype, indicating that PKD1 is dispensable for skin development and homeostasis. Upon wounding, however, PKD1-deficient mice exhibited delayed wound re-epithelialization correlated with a reduced proliferation and migration of keratinocytes at the wound edge. These results revealed a critical proproliferative role for PKD1 in epidermal adaptive responses, and authors suggested a potential therapeutic role of the kinase in skin wound and cancer treatment.[86,87]

**Protein kinase D1: inflammatory skin diseases**

We could not find data concerning PKD1 expression, participation and function of the kinase in inflammatory response and inflammatory skin diseases. In recent report Rashel *et al.* generated mice with targeted deletion of PKD1 in epidermis to evaluate the significance of PKD1 in normal and hyperplastic conditions, as mentioned above. In addition, the hyperplastic and inflammatory responses to topical phorbol ester were significantly suppressed suggesting involvement of PKD1 in tumor promotion (and inflammation). Consistently, when subjected to two-stage chemical skin carcinogenesis protocol, PKD1-deficient mice were resistant to papilloma formation when compared to control littermates.[87] However, similar to PKCε overexpression this could promotes the formation of highly metastatic squamous cell carcinomas (papilloma independent carcinomas—SCC).[88,89,90,91] PKCε is an activator of PKD1, although it influences many other cell processes.[92]

The only data in the literature have shown increased PKD1 expression in psoriatic lesions[11] and in hTert normal human keratinocytes (called also N/Tert-1 and N-hTERT,[10,27] stably transfected (infected) with amphotropic retroviral vectors encoding hTERT (catalytic subunit of telomerase),[93] thought to be activity-limiting component of the telomerase holoenzyme. hTERT is expressed only in germ cells, stem cells of renewal tissues and in cancer cells (including BCC and SCC). Telomerase activity is not detected in most somatic tissues (the proliferative basal layer of epidermis expresses telomerase, but anchorage-deprived cells underwent rapid loss of telomerase activity). The hTert(+) cells have a normal karyotype and the cells have undergone more than 80 population doublings (PDs) after hTert retroviral transduction, while control cells exhibit senescence-associated proliferation arrest after 8 PDs. Such immortalized cell typically has identifiable defects in $p16^{INK4a}$ expression, but retains functional p53. Keratinocytes that express hTERT and also acquire a defect in triggering $p16^{INK4a}$ expression become immortalized, but otherwise display normal growth characteristics and differentiation potential.[93] Increased expression of hTert together with early mutation in $p16^{INK4a}$ led us to the conclusion that hTert keratinocytes resemble more PMDs (premalignant diseases) and could be considered as a PMD cell line.[32]

The probable participation of PKD1 in inflammational skin process, diseases, and premalignant disorders was recently discussed from us in: [32] In brief:

Increased expression of hTERT is an early event in the pathogenesis of hyperproliferative skin diseases, overexpressed hTERT (under the control of NF-κB) is considered as a proproliferative (proinflammatory) marker, rather than cancer marke.[32] Our previous results in hTert keratinocytes showed not only increased expression of PKD1 (near ninefold),[10] but also altered—prodifferentiative function of the kinase in this cell line.[94] Knockdown of PKD1 resulted in inhibition of hTert (N/Tert-1) keratinocytes differentiation (decreased expression of K10 and involucrin — early differentiation markers) [94] and in downregulation of the expression (and activity) of EGFR and ERK1/2,[69] signal pathways closely connected with inflammation (see below) and oncogenesis[27,32]. These alterations were consequence only of increased hTert expression and spontaneous defect in $p16^{INK4a}$ expression. [93,94,69] Although additional study is necessary, PKD1 inhibition would not be a successful strategy for the treatment of premalignant diseases (PMDs) with high PKD1

expression because of the inhibition of differentiation (downregulation of K10, Involucrin).

In addition, a common feature of chronic inflammatory skin disorders such as psoriasis, atopic dermatitis, and allergic contact dermatitis, is epidermal hyperplasia and thickening, a phenomenon attributed to leukocyte-derived cytokines such as tumor necrosis factor (TNF-α) and interferon (IFN-γ), which are potent inducers of EGF family growth factors and EGFR. In the course of T-cell-driven skin inflammatory diseases, activated Th1 lymphocytes infiltrating the dermis and the epidermis are the major source of IFN-γ and TNF-α. Among the various leukocyte subsets, Th1 lymphocytes dominate psoriatic and allergic contact dermatitis lesions, but they are present also in chronic atopic dermatitis. These cytokines initiate a program of increased keratinocyte expression of inflammatory mediators, including adhesion molecules, cytokines, and chemokines. In particular, prominent keratinocyte expression of CCL2 (monocyte chemoattractant protein 1, MCP-1), CCL5 (RANTES), CXCL8 (IL-8), and CXCL10 (IFN-γ-induced protein of 10kd, IP-10) is a common finding in T-cell-mediated skin diseases, and mediates the recruitment of T cells and other leukocyte populations in the skin.[95,96]

$1,25(OH)_2D_3$ induced the expression of monocyte/ macrophage differentiation marker CD14, and lipopolysaccharide binding protein CAP18, highlighting the role of keratinocytes in innate immunity. CD14 has previously been described as a vitamin D-responsive gene in HL-60 promyelocyticleukemic and SCC25 cells. The expression of IL1 receptor like 1 (IL1RL1; T1/ST2) gene is induced by $1,25(OH)_2D_3$ in keratinocytes and SCC 25 (spino cellular cells) cells. As IL1RL1 gene disruption leads to a defect in T-helper type 2 (Th2) differentiation, vitamin D-mediated induction of its expression suggests that the shift of balance from pathogenic Th1 (IL-2-, IFN-γ-, and TNF-α secreting T cells;[97] IL-6 and IL-8,[98] responsible for the exacerbation of the skin inflammation, to non-pathogenic (anti-inflammatory) Th2 (IL-4-, IL-5, and IL-10-producing T cells) phenotype in psoriasis by VDR ligands may involve a direct role of keratinocytes in Th2 differentiation.[97] $1\alpha,25(OH)_2D_3$ the natural ligand of VDR enhances expression of anti-inflammatory cytokine, within the psoriatic lesions, as well as the expression of its receptors in keratinocytes.[98]

Pastore *et al.* found that during their early response to TNF-α or IFN-γ (leukocyte and Th1 derived), keratinocytes release EGFR ligands including TGF-α, which induce EGFR autophosphorylation and as a consequence activate its signal transduction cascade. EGFR activation leads to the persistent induction of the classical MAPK pathway identified as ERK 1 and 2 (ERK1/2), which plays a fundamental role in the EGFR-driven control of epidermal proliferation. In contrast, the other major subgroups of the MAPK family, namely, p38α and β and the JNK/stress-activated protein kinase 1 and 2 (JNK1/2), are weakly activated by EGFR (HER1, ErbB1), whereas they are highly stimulated on exposure to TNF-α. EGFR modulates skin inflammation via an ERK1/2-dependent mechanism, by affecting some chemokine expression in keratinocytes. ERK1/2 is involved in the regulation of a number of potent proinflammatory chemokines in epidermal keratinocytes *in vivo*, and suggest that it is part of a homeostatic mechanism that tends to oppose skin inflammation.[96]

EGFR ligands provide the main extracellular signals for high steady-state ERK1/2 activity, which in turn drives intracellular proproliferation and prosurvival programs. Pastore *et al.* found that ERK1/2 activity strictly depended on EGFR signaling in skin keratinocytes, with selective EGFR inhibition suppressing constitutive ERK1/2 and preventing its activation by TGF-α or the proinflammatory cytokines TNF-α and IFN-γ. Of note, the authors uncovered the existence of an EGFR-mediated differential regulation of cytokine-driven chemokine expression in these cells, with higher levels of EGFR activation associated with enhanced CXCL8 (IL-8), but suppressed CCL2, CCL5, and CXCL10 expressions, whereas opposite events were registered when EGFR function was blocked. Impairment of ERK1/2 obtained either by EGFR or MEK1/2 inhibition was similarly associated with downregulated CXCL8 (IL-8) and enhanced TNF-α-driven CCL2, CCL5, and CXCL10 in skin keratinocytes.[96]

The same authors also found that ERK1/2, JNK1/2, or p38αβ selective inhibition each impaired AP-1 *trans*-activation, whereas JNK1/2 or p38αβ inhibition also reduced NF-κB *trans*-activation through IκBα-independent mechanisms. This last finding could help to explain current and previous evidence that JNK1/2 or p38αβ inhibition decreases the promoter activity of proinflammatory genes known to strictly depend on NF-κB *trans*-activation, such as CCL2 and CCL5. Only CXCL8 promoter activity could be impaired by ERK1/2 inhibition, confirming that ERK1/2- driven AP-1 *trans*-activation plays a relevant role in its transcription.[96]

The TNF-α pathway is an interesting candidate to participate in the PKCα-mediated inflammatory response. TNF-α is upregulated early in induced cutaneous neutrophilic inflammatory responses. Genetic deletion of IκBα produced a dermatitis characterized by intraepidermal neutrophilic abscesses and elevation of TNF-α levels in the skin. Although targeting TNF-α to the epidermis produced only a mild dermal infiltrate that did not reproduce the IκBα null phenotype, this may reflect an extraordinarily elevated TNF-α level not achieved as a physiological response. The TNF-α/NF-κB pathway then must be considered in the future evaluation of the inflammatory arm of the PKCα response.[99]

It would appear that PKCα controls two distinct responses in keratinocytes, one related to growth and viability that is AP-1-dependent (induction of apoptosis) and the other related to chemotaxis and inflammation that appears to be independent of AP-1.[99] This suggests that the intraepidermal inflammatory response to TPA is a specific consequence of PKCα

activation, and not a general response to cell killing, but it does not exclude the possibility that apoptosis is secondary to inflammation.[99] Because the proapoptotic activity of cutaneous PKCα is prevented by blocking the AP-1 pathway, the proximal affectors of apoptosis are likely to be AP-1-regulated genes. The expression of AP-1 factors is altered in neoplastic keratinocytes, and this could contribute to the differences in response to PKCα activation between normal and neoplastic keratinocytes. Elevated PKCα activity in neoplastic cells (lack in BCC) could also contribute to the inflammatory response in cutaneous neoplasms. The authors cannot exclude a role for TNF-α as a mediator of the apoptosis observed in this model. TNF-α is PKC inducible and proapoptotic when overexpressed in mouse keratinocytes, and is highly induced in PKCα transgenic skin. However, TNF-α is not suppressed by A-FOS [*A-FOS* transgene (a dominant negative that abolishes AP-1 DNA binding)] in PKCα transgenic keratinocytes. Exogenous TNF-α cannot induce apoptosis in cultured PKCα keratinocytes.[100] Together, these results suggest that TNF-α is not the mediator of the apoptotic response, but further studies will be required to elucidate the AP-1-dependent pathways altering keratinocyte viability.[99]

TNF-α is a cytokine critical for the development of psoriasis and blockade of its activities with biological agents can lead to remarkable improvement of psoriatic skin lesions. PKCζ is also critically located in the transduction pathway from TNF-α to activate NF-κB, the event crucial for the inflammatory process within the psoriasis lesions. However, mitogen-activated protein kinase (MAPK–pERK and pJNK) pathway, known to be activated as a result of PKCζ signaling, was activated by such treatment (6 min after TNF-α stimulation) in KCs (keratinocytes) as well as in HaCaT keratinocytes (but not detected in psoriatic cells). PKCζ gene expression was assayed using a quantitative real-time PCR in six pairs of psoriasis plaques and uninvolved skin, and was found to be increased significantly in all the psoriasis samples compared with the corresponding uninvolved skin.[101] PKCζ activity is required for EGF-induced extracellular signal-regulated kinase (ERK) activation in both normal human adult epidermal keratinocytes and five of seven SCCHN (Spino cellular carcinoma head and neck) cell lines.[102]

PKCζ is required for TNF-α signaling and nuclear factor-κB (NF-κB) activation. [101] PKCζ phosphorylates the IKKκ subunit *in vitro*, possibly through their direct interaction.[103]

In psoriatic epidermis are detected mutations of CARD14, encoding a nuclear factor of kappa light chain enhancer in B cells (NF-κB) (epidermal regulator of NF-κB), within skin epidermis and increased expression and activity of EGFR, STAT3, PKD1, NF-κB, hTERT, and other kinases.[32]

Uninvolved psoriatic skin showed a similar pattern of predominant PKD protein expression in the stratum basalis, but in contrast, involved (lesional) psoriatic epidermis demonstrated that a diffuse pattern of staining with ectopic foci of increased staining intensity in the suprabasilar layers was observed. This result is consistent with altered PKD protein expression in the epidermis potentially mediating the antidifferentiative phenotype characterizing psoriasis.[32,11] In addition, 14-3-3σ mRNA expression was increased in psoriasis and contact dermatitis, but not in BCC. In atopic dermatitis, no significant difference between involved and uninvolved skin was found. Only 14-3-3τ expression (protein) was significantly increased in involved psoriatic skin compared with uninvolved skin.[104] The docking interaction between 14-3-3τ and PKD1 actually decreases PKD1 catalytic activity, probably through nuclear export of activated PKD1.[7]

In psoriatic epidermis, the EGFR is overexpressed not just in the basal layer, but in all nucleated strata of the epidermis, consistent with the suprabasal proliferation that occurs in this disease. Furthermore, both TGFα and AR are found at elevated levels throughout the nucleated layers of psoriatic epidermis. Direct support for a role of EGFR activation in the development of skin tumors comes from studies in transgenic mice, in which overexpression of TGF-α targeted to the epidermis elicits hyperplasia, hyperkeratosis, papillomas, and squamous cell carcinomas.[105,106] EGF ligands and EGFR activation could also activate PKD1 (called also PKCμ), which consequently could activate ERK1/2 and NF-κB, participating in skin inflammatory responses, similar to PKCζ. As it was described the above stimulation of human colonic epithelial NCM460 cells with the GPCR agonist and bioactive lipid lysophosphatidic acid (LPA) led to a rapid and striking activation of PKD2, the major isoform of the PKD family expressed by these cells. LPA stimulated the production of interleukin 8 (IL-8), a potent proinflammatory chemokine, and stimulated NF-κB activation. PKD2 gene silencing dramatically reduced LPA stimulated NF-κB promoter activity and IL-8 production. These results imply that PKD2 mediates LPA-stimulated IL-8 secretion in colon NCM460 cells through an NF-κB dependent pathway.[2] Thus, the inflammatory answer can be stimulus (TLR)-dependent, cell type (signal transductional pathway), and disease specific, leading to production of different cytokines, including adhesion molecules, ILs, and chemokines and PKD1 could participate in this process. PKCζ is also crucial for macrophage activation and expression of adhesion molecule ICAM-1, and metalloproteinase-9 (MMP-9),[101] through ERK1/2 Snail pathway but not p38.[19,107,108,109] MMP-9 was detected in the epithelium in both chronic wounds (chronic leg ulcers)[110] and found in papillomas.[111] MMP-9 expression has been reported recently in dyskeratotic foci of Bowen's disease and in infiltrative edges of microinvasive carcinomas,[112] SCC,[112,110,111], and BCC.[112,113]

In addition, MMP-9 was found to be elevated in the Oral Lichen Planus (OLP) inflammatory infiltrating cells[114] in tissue, serum and saliva samples of oral PMDs than in healty controls (distinguish oral leucoplakia and Oral SCC from healthy control).[115]

Moreover, downregulation of E-cadherin and β-catenin expressions was found in the granular layer and basal layer of the psoriatic lesions. Downregulation of E-cadherin and β-catenin expressions and increased nuclear β-catenin, cyclin D1 overexpression in psoriatic skin are probably involved in keratinocyte hyperproliferation in psoriasis vulgaris compared with uninvolved or normal skin.[116,117] Increased active unphosphorylated β-catenin was also detected within the differentiating compartment of involved psoriatic epidermis.[32,118]

Calcitriol (vitamin $D_3$, $1,25(OH)_2D_3$) and its analogs attenuate epidermal inflammation and inhibit the hyperproliferation of keratinocytes associated with the inflammatory disorder, psoriasis. Since activation of extracellular signal regulated kinase (ERK) promotes keratinocyte proliferation and mediates epidermal inflammation, the effect of calcitriol on ERK activation in HaCaT keratinocytes exposed to the ubiquitous inflammatory cytokine TNF was studied. Ziv *et al.* established that TNF activated ERK in an EGFR and Src-dependent and EGFR- and Src-independent modes. EGFR-dependent activation resulted in the upregulation of the transcription factor, c-Fos, while the EGFR-independent activation mode was of a shorter duration, did not affect c-Fos expression, but induced IL-8 mRNA expression. Calcitriol, enhanced TNF-induced EGFR-Src-dependent ERK activation and tyrosine phosphorylation of the EGFR, but abolished the EGFR-Src independent ERK activation. These effects were mirrored by enhancement of c-Fos and inhibition of IL-8 induction by TNF. Treatment with calcitriol increased the rate of the de-phosphorylation of activated ERK, accounting for the inhibition of EGFR-Src independent ERK activation by TNF. It is possible that effects on the ERK cascade contribute to the effects of calcitriol and its synthetic analogs on cutaneous inflammation and keratinocyte proliferation.[119,120]

Vitamin D-mediated regulation of c-fos expression was confirmed by Q-PCR, where 6 h treatment of KerTr (immortalized keratinocytes) and NHEK (normal human epidermal keratinocytes) with $1,25(OH)_2D_3$, showed 3–fivefold induction in its expression. Its expression was also induced at 24 and 48 h timepoints in KerTr cells.[97] AP-1 factors consist of homo- or heterodimers of jun (c-jun, junB, junD) and fos (Fra-1, Fra-2, c-fos, fosB) family members, and depending on the dimer composition, these factors can function as either activators or suppressors of transcription. The AP-1-binding sites of different promoters are not identical and may bind different AP-1 homo- and heterodimers, which in turn could account for their different regulation.[121] Furthermore, c-fos expression is reduced in psoriatic lesional skin compared with normal epidermis. Therefore, induction of c-fos by $1,25(OH)_2D_3$ may help in normalization of differentiation observed by the treatment of psoriatic lesions by VDR ligands.[97]

Crucially, the expression kinetics of immediate early genes (IEG)-encoded protein products, such as c-Fos, is marked in response to agonists that induce sustained rather than transient ERK and RSK activation.[122] c-Fos protein is very unstable (half-life, $\tau 1/2 \approx 30$ min) and will accumulate only if its C terminus is phosphorylated under conditions of sustained ERK activation. Because c-Fos is an important component of the dimeric AP-1 transcription factor, an increase in its stability results in greater promoter occupancy and expression of target genes[122] (Murphy and Blenis, unpublished) and in cellular transformation. Accordingly, expression of the late-response gene Fra-1, a target of c-Fos, is sustained only after prolonged expression of c-Fos. Thus, the behavior of c-Fos after transient or sustained ERK signaling enables the cell to distinguish among agonists that induce different activation kinetics. Therefore, by its ability to exist in unstable and stabilized states, the c-Fos transcription factor can function as a 'sensor' for ERK activation dynamics. [122]

Sustained activation of ERK signaling is involved in cell-cycle progression, cellular transformation and differentiation. After ligands bind to the receptor tyrosine kinases (RTKs) and Ras is activated, members of the Raf family are recruited to membrane-associated activators. Raf then activates mitogen-activated protein kinase kinases 1 and 2 (MEK1/2), which in turn phosphorylate and dissociate from ERK1/2. Activated ERK1/2 phosphorylate targets at the membrane and in the cytoplasm, such as ribosomal S6 kinases (RSKs), and a portion of active ERK1/2 translocates into the nucleus by an unknown mechanism. Active RSK1/2 also translocate into the nucleus, where together with nuclear RSK isoforms (RSK3/4, MSK1/2) and ERK1/2, they phosphorylate and activate several nuclear targets such as the transcriptional regulators Ets, STAT, CREB, and histone H3. This results in the rapid induction of immediate early genes (IEGs) including c-fos. If signaling remains active after the IEG-encoded protein products are translated, the sustained signal can lead directly to phosphorylation of the IEG products and can prolong their expression and activity in the nucleus for several hours. If signaling is transient, the IEG products are unstable and are degraded by the proteasome. [122] In addition to increasing stability, the C-terminal modification of c-Fos enhances the process of ERK mediated phosphorylation of $Thr^{325}$ and $Thr^{331}$ within its C terminus. On the basis of these observations, the authors propose that the DEF domain and phosphorylation of $Thr^{325}$ and $Thr^{331}$ are required to drive c-Fos into an activated state (during sustained signaling), necessary for keratinocyte differentiation.[122] Recent reports proved the participation of ERK1/2 in keratinocytes differentiation,[123] connected with filaggrin expression.[124]

Inflammation and proliferation are regulated by a plethora of transcription factors, with nuclear factor-κB (NF-κB) considered to be a master regulator of these processes. NF-κB is also important in the development, prevention and therapy of cancer. NF-κB activity is stimulated by many pathways that converge on IκB kinases, including the signaling pathways activated by various cytokines, such as the proinflammatory

cytokine IL-1, lipopolysaccharide (LPS), and tumor necrosis factor α (TNF-α). In mammals, the NF-κB family of proteins includes NF-κB1 (p105 processed to p50), NFκB2(p100 processed to p52), RelA (p65), RelB, and cRel. A crucial negative regulator that controls NF-κB activation is the inhibitor of κB (IκB), which binds to p65 in the cytosol to block the nuclear translocation of the p65/p50 heterodimer. Phosphorylation of IκB by activated IκB kinase (IKK)2 initiates the ubiquitylation and eventual proteasomal degradation of IκB, and a direct consequence of IκB degradation is nuclear entry of p65/p50 to transactivate specific gene expression. Thus, IKK plays an essential role in NF-κB activation. The kinase activity of IKK depends on the formation of the IKK complex by the IKKα, β, and γ subunits, which is activated upon phosphorylation by growth factors, proinflammatory cytokines (such as TNFα), and hormones through the TNF receptor or toll-like receptor superfamily. IKK also phosphorylates p65 to promote its activity.[125]

NF-κB-regulated genes play important roles in inflammation, immunity, cell growth, and cell survival. NF-κB activation is mediated throughthe activation of specific IκB kinases (IKKs) and the subsequent phosphorylation of IκB.[126]

1,25-Dihydroxyvitamin D ($1,25(OH)_2D_3$) is known to suppress NF-κB activity, but the underlying mechanism remains poorly understood. Vitamin D receptor (VDR) physically interacts with IκB kinase β (IKKβ) to block NF-κB activation. $1,25(OH)_2D_3$ rapidly attenuates TNFα- induced p65 nuclear translocation and NF-κB activity in a VDR-dependent manner. VDR overexpression inhibits IKKβ-induced NF-κB activity. GST pull-down assays and coimmunoprecipitation experiments demonstrated that VDR physically interacts with IKKβ and that this interaction is enhanced by $1,25(OH)_2D_3$. This interaction was not altered substantially by the presence of $1,25(OH)_2D_3$, consistent with the above observation that, at high concentrations, VDR suppressed NF-κB even in the absence of $1,25(OH)_2D_3$. Protein mapping reveals that VDR-IKKβ interaction occurs between the C-terminal portions of the VDR and IKKβ proteins. Reconstitution of $VDR^{-/-}$ cells with the VDR C terminus restores the ability to block TNFα-induced NF-κB activation and IL-6 upregulation. VDR-IKKβ interaction disrupts the formation of the IKK complex and, thus, abrogates IKKβ phosphorylation at $Ser^{177}$ and abolishes IKK activity to phosphorylate IκBα. Consequently, the stabilization of IκBα arrests p65/p50 nuclear translocation. Together, these data define a novel mechanism, whereby $1,25(OH)_2D_3$- VDR inhibits NF-κB activation in HEK293 (human embryonic kidney; the cells contained Adenovirus 5 DNA, forms tumors in nude mice) and RAW264.7 (mouse; Abelson murine leukemia virus-induced tumor).[125]

Since nuclear factor-κB (NF-κB) plays a pivotal role in the regulation of cell proliferation, differentiation, and apoptosis, the authors examined the capability of 20-hydroxycholecalciferol to modulate the activity of NF-κB, using 1,25-dihydroxycholecalciferol (calcitriol) as a positive control. 20-hydroxycholecalciferol inhibits the activation of NFκB DNA-binding activity as well as NF-κBdriven reporter gene activity in keratinocytes. In addition, 20-hydroxycholecalciferol-induced significant increases in the mRNA and protein levels of the NF-κB inhibitor protein, IκBα, in a time-dependent manner, while no changes in total NF-κB-p65 mRNA or protein levels were observed. Another measure of NF-κB activity, p65 translocation from the cytoplasm into the nucleus was also inhibited in extracts of 20-hydroxycholecalciferol treated keratinocytes. Increased IκBα was concomitantly observed in cytosolic extracts of 20-hydroxycholecalciferol treated keratinocytes, as determined by immunoblotting and immunofluorescent staining. In keratinocytes lacking vitamin D receptor (VDR), 20-hydroxycholecalciferol and $1,25(OH)_2D_3$ did not affect IκBα mRNA levels, indicating that it requires VDR for its action on NF-κB activity. Comparison of the effects of calcitriol, hormonally active form of vitamin D3, with 20-hydroxycholecalciferol shows that both agents have a similar potency in inhibiting NF-κB and also increases IκBα protein levels through the induction of IκBα mRNA expression (no statistically significant difference), forming transcriptionally inactive NF-κB/IκB complexes. Since NF-κB is a major transcription factor for the induction of inflammatory mediators, these findings indicate that 20-hydroxycholecalciferol, calcitriol and its analogs may be an effective therapeutic agent for inflammatory and hyperproliferative skin diseases. Since recent studies demonstrate that the activation of the alternative NF-κB pathway can also lead to the translocation of p65-containing dimers into the nucleus (including PKD1), the authors data cannot exclude the possibility that $20(OH)D_3$ also blocks this signaling pathway as well. Vitamin D analogs are now widely used drugs for the treatment of psoriasis, an inflammatory hyperproliferative dermatoses and cancer.[119,127,126]

The side chain of vitamin D3 is hydroxylated in a sequential manner by cytochrome P450scc (*CYP11A1*) to form 20-hydroxycholecalciferol, which can induce growth arrest and differentiation of both primary and immortalized epidermal keratinocytes. A new pathway for the metabolism of vitamin D and pro-vitamin D that is catalyzed by cytochrome P450scc (*CYP11A1*), the enzyme catalyzing the conversion of cholesterol to pregnenolone for steroid hormone synthesis. The hydroxyl group of $20(OH)D_3$ is attached at the C20 position, which is interesting, since the attachment at C1 is considered to be required for full biological activity and calcemic effects. $20(OH)D_3$ could have systemic effects when produced in organs expressing high levels of P450scc, such as adrenal cortex, corpus luteum, follicles, and placenta, and skin expresses low levels of P450scc. Moreover, $20(OH)D_3$ has a significant biological activity in human keratinocytes, as it inhibits their proliferation and stimulates their differentiation.[126]

NF-κB plays an important role in protecting keratinocytes against apoptosis during programmed

cornification. In normal human keratinocytes, $1,25(OH)_2D_3$ reduces NFκB DNA-binding activity by increasing IκBα protein levels, which inhibits IL-8 production. A similar effect is also seen in murine macrophages. Effects of $1,25(OH)_2D_3$ on NF-κB that are not mediated by the VDR have also been reported for fibroblasts lacking the VDR. [126]

Inhibitors targeting the NF-κB-signaling pathway effectively suppress NF-κB activity, protect and relieve inflammatory symptoms, and induce apoptosis of tumor cells. NFκB represents an attractive drug target for therapy of inflammatory and auto-immune disorders, as well as for cancer. Thus, $20(OH)D_3$ is a new powerful analog of vitamin D3 and could exert beneficial effects in inflammatory, autoimmune disorders, and cancer. [119,127,126]

As it was mentioned above, VDR (Vitamin D receptor) ligands inhibit the expression of proinflammatory cytokines produced by T lymphocytes, such as IL-2, IFN-γ, IL-6, and IL-8 (NF-κB-binding sequence), which are responsible for the exacerbation of the skin inflammation. Apart from that, $1α,25(OH)_2D_3$ enhances the expression of anti-inflammatory cytokine, IL-10, within the psoriatic lesions, as well as the expression of its receptor in keratinocytes.[98]

In other tissues, $1,25(OH)_2D_3$ downregulates also a variety of genes, including IL-12, IL-8 (induced by MMP-8 in breast cancer), MCP-1 (CCL2), PAI-1, angiotensinogen, and microRNA-155 by blocking NF-κB activation. Therefore, $1,25(OH)_2D_3$ suppression of NF-κB activation has great biological and pathological relevance,[125] and could be used in the treatment not only of skin inflammatory and premalignant diseases, but also in the treatment of skin and Head and Neck cancer (SCC and BCC)[119,127,128,97,31] (see below).

Moreover, as it was mentioned above, mTOR major upstream and downstream regulator gene expression was assessed in skin biopsies from 15 patients affected by psoriasis, 5 patients with allergic contact dermatitis (ACD), 5 patients with atopic dermatitis (AD), and 3 patients with EGFR-inhibitor-induced skin rash. All analyzed skin diseases showed an increase of mTOR gene expression, whereas mTOR upstream negative regulators were reduced or not enhanced in all of them. mTOR was strongly expressed in all epidermal layers of lesional and non-lesional psoriatic skin. Conversely, proinflammatory conditions, *in vitro*, were not able to increase mTOR levels, except for UVB. Similarly, anti-TNF-α therapy was not able to reduce mTOR gene expression in patients with psoriasis. Balato *et al.*'s study provides evidence that mTOR is involved in cutaneous inflammatory process, but through a signaling not directly dependent from Thl to Th17 pathway.[47]

The 14-3-3 proteins are known to shuttle phosphorylated proteins among different subcellular locations. Among epithelial tissues, the epidermis is characterized by the highest level of 14-3-3σ expression [called also stratifin (SFN)]. Keratinocytes which exit from the stem-cell compartment and differentiate show an increase in 14-3-3σ expression, suggesting that 14-3-3 may be linked to terminal differentiation of epithelial cells.[129] In primary human keratinocytes, inactivation of 14-3-3σ results in immortalization. [129] How the loss of 14-3-3σ contributes to immortalization is not clear. Lodygin *et al.* studied *in vivo* expression of 14-3-3σ protein in several skin diseases, which are characterized by hyperproliferative keratinocytes. Unexpectedly, the 14-3-3σ protein was expressed at high levels in psoriasis (11 of 11 patients), condylomata acuminata (11/11), actinic keratoses (11/11), and squamous cell carcinomas (SCC) (11/11), equal to that of normal epidermis. However, keratinocytes that had undergone transformation to basal cell carcinoma (BCC) showed partial (10 of 41; 24.4%) or complete (19 of 41; 46.3%) loss of 14-3-3σ protein expression. BCC (5/5), SCC (6/6), and actinic keratoses (7/7) concomitantly expressed the p53-homolog p63 and 14-3-3 at high levels, ruling out potential inhibitory effects of p63 isoforms on 14-3-3σ transcription as the basis for loss of 14-3-3σ expression. Of 41 BCC samples isolated by laser-capture microdissection, 28 (68.3%) showed CpG hypermethylation of the 14-3-3σ promoter combined with reduced or absent 14-3-3σ protein levels in 22 cases (78.6%). Since it has been reported that BCC retain wild-type $p16^{INK4A}$ [overexpressed in dysplasia and mutated (mostly hypermethylated) in premalignant disease (PMDs) and SCC[27,129,130] and there BCC with CpG methylation of 14-3-3σ did not show CpG methylation of $p16^{INK4A}$ (0/17), silencing of 14-3-3σ may contribute to evasion of senescence in BCC.[129] As it was mentioned above, PKD1 phosphorylates Ser11 (S11) on transcription factor Snail, triggering nuclear export of Snail via 14-3-3σ binding in prostate cancer.[131] The main function of Snail is inhibition of E-cadherin expression liberation of active β-catenin and EMT induction.[132] Snail protein is also pulled down with 14-3-3η independent of PKD1.[131] Activity of this minimal promoter and Snail RNA levels were dependent on ERK-signaling pathway. NFκB/p65 also stimulated Snail transcription through a region located immediately upstream the minimal promoter, between –194 and -78, [132] and GLI 1.[133]

In mature epithelium, the expression of p63 (p53 family member) is highest in basal epithelial cells, where it functions as an inhibitor of NOTCH1 expression, and becomes downregulated during terminal differentiation coincident with NOTCH1 upregulation. Reactivation of p63 expression is observed in the suprabasal layers of dysplastic mucosa, and overexpression and/or genomic amplification of the TP63 locus is observed in the majority of invasive HNSCCs.[53] In the stratified epithelium, Notch has a central role in promoting terminal differentiation, negatively regulated by EGFR, which is mediated through both direct effects (e.g., on activation of suprabasal keratins) and indirect effects on the Wnt, hedgehog, and interferon response pathways. [53]

Alterations in genes and pathways that regulate cellular signaling, cell cycle, proliferation, differentiation, apoptosis, genomic stability, motility, angiogenesis, and metastasis are significantly

associated with development and progression of a potentially malignant disorder to OSCC. Aberrant expression and function of molecules involved in these signaling networks have been considered as biomarkers for risk assessment of malignant transformation. These biomarkers include increased expression of hTERT (human telomerase catalytic protein subunit), abnormalities in expression of the EGFR and/or its ligands epidermal growth factor (EGF), tumor growth factor-alpha (TGF-α), heparin-binding EGF (HB-EGF), and amphiregulin (AR) are common features of hyperproliferative (PMDs) and neoplastic epithelia.[27,32,105,106] Upregulated NF-kB closely connected with increase in cyclooxygenase-2 (COX-2; PGHS-2—prostaglandin G/H synthase) expression.[134] COX-2 is a target gene of NF-kB and COX-2 stain was found increasing from hyperplasia to dysplasia and was highest in squamous cell carcinoma;[135,27,62] see below—Figure 8.

**Protein kinase D1 in skin and head and neck cancer**

Proves for the participations of PKD1 in inflammatory and tumor promoting events, in accordance with our results and hypothesis,[27,32] were published Chiou *et al.*[136] Topical application of TPA (A) or DMBA (B) over 12 h, according to (DMBA)-initiated and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumorigenesis in ICR mice, greatly increased the protein levels of PKD1 and CD34 (stem-cell marker), decreased ERK1/2, increased c-Myc, cyclin B1/CDK1 complexes, and Cdc25A. Pretreatment with AcEGCG (peracetylated (−) epigallocatechin-3-gallate) leads to the activation of ERK, the degradation of Cdc25A, and the inhibition of cyclin B1/CDK1 complex assembly; these effects cause G2/M phase arrest and block mitotic progression. Pretreatment with AcEGCG at a dose of 1 or 5 μM resulted also in a decrease in the levels of phosphorylated JNK1/2, p38, and PI3K/Akt compared with the levels in DMBA/TPA-mediated tumors (decreased p-ERK1/2 increased, p-PI3K, p-JNK1/2, and p-p38; and increased levels of p53, p21, and c-Myc (in papillomas)). The authors also observed that the DMBA/TPA stimulation of NF-κB, C/EBPs, and CREB-DNA-binding activity was attenuated by pretreatment with AcEGCG in a dose-dependent manner, which transcribe proinflammatory and proproliferative genes, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), ornithine decarboxylase (ODC), and vascular endothelial growth factor (VEGF). Overall, the authors speculated that AcEGCG exerts antiproliferative and/or anti-inflammatory effects in CD34+ skin stem cells and skin tumors and that the suppression of PKD1 activity and its downstream signaling pathways may be involved in the prevention of skin carcinogenesis.[136]

In this study, Chiou *et al.* also analyzed CD34 and PKD1 expression levels in human keratinocytes (HaCaT) and human epithelial carcinoma cells (A431) and found that A431 cells had an ~ fourfold induction of CD34 and PKD1 expression compared with HaCaT cells. In addition, CD34 and PKD1 upregulation correlated with a fast proliferative potential. Their results strongly suggest that the overexpression and activation of PKD1 in CD34+ skin stem cells and skin tumors are potential targets for the treatment of skin carcinogenesis.[136] De Craene *et al.* results indicate that enhanced Snail expression, Snail transgenic mice, contributes to the stabilization, expansion, and survival of skin stem cells *in vivo* (CD34+[137]), which can result in both skin tumor initiation and malignant progression for a variety of epithelial-derived tumor types, such as basal cell carcinoma, squamous cell carcinoma, and sebaceous gland carcinoma.[138] In addition, when subjected to two-stage chemical skin carcinogenesis protocol, PKD1-deficient mice were resistant to papilloma formation when compared to control littermates.[87] Ristich *et al.* do not detect PKD1 in SCC, and probably, downregulation of its expression could be accepted as a critical point in progression of benign tumors to SCC[11] (low expression as a cancer marker), and two-stage carcinogenesis protocol causes an oncogenic mutation in the *H-Rras* gene and appeared to be ideal for studing physiological responces to alterations in gene expression against oncogenic Ras signaling in living animals,[139] leading to the development of SCC. Recently, Zhang *et al.* detected significantly lower PKD1 expression in as compared to the normal and adjacent normal tissues, as a result of combination of genetic and epigenetic alterations. The results obtained from cBioPortal analysis of 530 HNSCC tumors from TCGA showed low level of DNA methylation on *PRKD1* gene (see below).[140]

The Chiou *et al.*'s article is interesting with that that it is the only paper detecting PKD1 effects in mouse keratinocytes on different key kinases. Increased PKD1 levels, after two-step carcinogenetic protocol, resulted in decreased p-ERK1/2, and increased p-PI3K, p-JNK1/2, p-p38. The decreased in p-ERK1/2, decreased activity of ERK1/2 is in accordance with our results, which also showed that PKD1 inhibited phosphorylation of ERK1/2 in normal human keratinocytes,[17,16] unpublished data with PKD1 anti-sense oligonucleotide] connected with stimulation of keratinocytes proliferation, contrarily to expected stimulation of ERK1/2 according to scientific data (PKD1—substrates and function). In contrary in hTert (called also N/ Tert-1 and N-hTERT,[10,27]), keratinocytes PKD1 levels are near ninefold higher compared with normal keratinocytes,[10] and the kinase induces ERK1/2 and EGFR expression.[86] According to the same sources, increased PKD1 levels has to inhibit phosphorylation (activity) of PI3K, JNK1/2, and p38 in other cell types, but Chiou *et al.* detected the reversed effects. The authors also proved NF-κB activation by increased PKD1 levels in mouse keratinocytes in the process of tumor promotion.[136]

Till recently the only research in field of Head and Neck SCC (HNSCC) explored the expression of PKD isoforms was evaluated in a panel of nine head and neck cancer cells, including Cal33, UMSCC-1, UMSCC-10A, UMSCC22B, UPCI 4B, UPCI 15B, OSC19, 686LN, and 1483. Het-1A, a normal human esophageal squamous epithelial cell line, was included as a control.

PKD1 protein levels were significantly lower in all of the HNSCC cell lines examined as compared to the control Het-1A cells. In contrast, levels of PKD2 protein were similar or slightly higher in HNSCC cell lines than in Het-1A with the exception of three lines showing reduced PKD2 expression (UMSCC-1, UMSCC22B, and 1483). PKD3 was minimally expressed in the control and in almost all HNSCC cell lines examined. A similar trend was found in the transcript levels of PKD1 and 2. PKD1 transcript levels were significantly lower in all 9 HNSCC cell lines as compared to that in Het-1A. In addition, levels of PKD2 were higher in UMSCC-10A, UPCI 4B, UPCI 15B, and OSC19, and lower in Cal33, UMSCC-1, UMSCC22B, 686LN, and 1483 cells, which correlates to the protein levels of PKD2 found in these cell lines. Taken together, among members of the PKD family, PKD1 was the only isoform, whose protein and transcript levels were persistently downregulated in HNSCC cell lines, as compared to the normal and adjacent normal tissues.[140]

There was no significant correlation between PKD1 expression and pathologic grade or depth of primary tumor invasion (T status), neither was there significant association with age and gender. In normal squamous mucosa, strong membranous pattern of PKD1 staining as well as diffused or granular cytoplasmic staining of PKD1 were observed, which is in contrast to the weak and diffused cytoplasmic staining of PKD1 in tumor tissues. Interestingly, in a small cohort of metastases (15 lymph node metastases and one liver metastasis), a significant reduction of PKD1 expression was observed in distal metastases as compared to the localized primary tumors ($p = 0.002$), suggesting a reverse correlation with tumor metastasis. In contrast, transcript levels of PKD2 and PKD3 showed the opposite trend, i.e., increased PKD2 or PKD3 expression in tumor *vs.* normal tissue. PKD1 mRNA was downregulated in 87% HNSCC tumors (459 out of 530 cases with over twofold mRNA downregulation).[140]

An analysis of 530 HNSCC tumors from the TCGA via cBioPortal demonstrated low levels of DNA methylation on PRKD1 gene. Further analysis indicated 13% cases (67 out of 530 cases) of PKD1 had loss of heterozygosity (LOH), while only three cases (< 1%) of PKD1 showed homozygous deletion. Thus, a combination of genetic and epigenetic alterations contributed to the downregulation of PKD1 expression.[140]

PKD1 was induced upon Dox (doxycycline) treatment, but the expression and activity of PKD1 were not essential for the proliferation, migration, or invasion of HNSCC cells. Dox-treated PKD1-c1 clones (positive PKD1 clone) also showed elevated p-EKR1/2 and reduced IκBα, indicative of the activation of the MEK/ERK1/2 and the NF-κB-signaling pathways. In contrast, the PI3K/Akt-signaling pathway was not affected, since p-Akt level was not altered. Accordingly, IHC staining showed increased cell proliferation (Ki67) in tumor explants of the Dox-treated PKD1-c1 group as compared with the controls. Thus, the overexpression of PKD1 promoted the growth of HNSCC tumor xenografts.[140]

The authors have made also very interesting analysis connected with reexpression of PKD1 in tumors with downregulated *PRKD1*. Zhang *et al.*'s data consistently showed that either knockdown or overexpression of PKD1 did not significantly alter the proliferation of HNSCC cells *in vitro*. However, interestingly, the induction of PKD1 *in vivo* by Dox provided a slight growth advantage to the HNSCC tumor xenografts and resulted in a significant increase in the final tumor weight in Dox-induced *vs.* the non-induced tumors. This correlated with increased ERK1/2 and NF-κB-signaling activity, and enhanced tumor-cell proliferation *in vivo*. Later, they demonstrated that in the presence of a mitogen (bombesin or GRP) that activates PKD, overexpression of PKD1 potentiated the mitogenic effects of bombesin in HNSCC, and depletion of endogenous PKD2, the predominant PKD isoform expressed in HNSCC cells, abolished such effect. At molecular level, overexpression of PKD1 promoted bombesin- or GRP-induced ERK1/2 activation, while knockdown of PKD2 reduced EKR1/2 activation. It has been shown that the mitogenic effects of GRP are mediated by the activation of the MEK/EKR1/2 MAPK pathway through transactivating EGFR in HNSCC cells. The authors' findings imply that PKD1 and PKD2 may contribute to the mitogenic effect of GRP and bombesin by facilitating the activation of ERK1/2. PKD2 mRNA was upregulated in seven out of ten tumors *vs.* normal in patient-paired HNSCC tissue specimens. Thus, it is possible that PKD2 plays a predominant role in the growth, survival, and motility of HNSCC cells, and these functions have compensated the loss of PKD1 in tumors, and their data from PKD2-knockdown cells support this claim.[140]

Although, according Wang JN *et al.* inhibiting the expression of PKD1 in SCC-25 cells by RNA interference could inhibit the growth and promote the apoptosis of SCC-25 cells via downregulating Bcl-2 expression. Additionally, inhibiting PKD1 expression could downregulate the expression of P-gp, thereby decreasing both the IC50 and resistance index of paclitaxel. The authors concluded that PKD1 plays an important role in regulating the biobehavior of SCC-25. It is a potential therapeutic target for oral squamous carcinoma.[141]

Low pH and hypoxia are unique characteristics of the tumor microenvironment. The aim of a study was to investigate the role and mechanism of PKD1 in regulating metabolism in the human tongue squamous cell carcinoma (TSCC) cell line SCC25 under a hypoxic condition, as well as growth and apoptosis. Chen J *et al.* found that hypoxia not only induced the expression of HIF-1α, but also induced the expression and activation of PKD1. Moreover, the authors inhibited the expression of PKD1 by shRNA interference, and the growth of SCC25 cells under hypoxia was significantly decreased, as well as the expression of HIF-1α, while the percentage of apoptotic SCC25 cells was increased. Furthermore,

stable silencing of PKD1 in SCC25 cells under a hypoxic condition decreased glucose uptake, lactate production and glycolytic enzyme (GLUT-1 and LDHA) expression, as well as reduced the phosphorylation of p38 MAPK. The results revealed that following inhibition of the expression of PKD1 under a hypoxic condition, the growth and metabolism of the SCC25 cells were significantly suppressed. In contrast, when PKD1 was overexpressed in SCC25 cells, the results were completely reversed, except for growth and apoptosis. Taken together, the authors results demonstrated that PKD1 not only regulates the hypoxic glycolytic metabolism of cancer cells via regulation of the expression of HIF-1α and glycolytic enzymes, but is also involved in the remodeling of the acidic tumor microenvironment. They found that PKD1 is associated with the activation of p38 MAPK signaling and the activation of p38 MAPK signaling is necessary for HIF-1α accumulation and nuclear translocation. This study suggests that PKD1 may be a potential target for microenvironment-directed tumor biotherapy.[142]

Integrin recycling is critical for cell migration. Protein kinase D (PKD) mediates signals from the platelet-derived growth factor receptor (PDGF-R) to control αvβ3 integrin recycling. We now show that Rabaptin-5, a Rab5 effector in endosomal membrane fusion, is a PKD substrate. PKD phosphorylates Rabaptin-5 at Ser407, and this is both necessary and sufficient for PDGF-dependent short-loop recycling of αvβ3, which in turn inhibits α5β1 integrin recycling. Rab4, but not Rab5, interacts with phosphorylated Rabaptin-5 toward the front of migrating cells to promote delivery of αvβ3 to the leading edge, thereby driving persistent cell motility and invasion that is dependent on this integrin. Consistently, disruption of Rabaptin-5 Ser407 phosphorylation reduces persistent cell migration in 2D and αvβ3-dependent invasion. Conversely, invasive migration that is dependent on α5β1 integrin is promoted by disrupting Rabaptin phosphorylation. These findings demonstrate that the PKD pathway couples receptor tyrosine kinase signaling to an integrin switch via Rabaptin-5 phosphorylation.[242, 79]

Analysis of basal cell carcinoma (BCC) lesions of Ristich *et al.* showed increased expression of PKD1 (PKCμ) when compared with normal epidermis, but not in SCC lesions (squamous cell carcinoma). For BCCs samples in which comparison was possible, the tumors exhibited elevated PKD immunoreactivity relative to the basal layer of the normal epidermis. This result suggests that BCCs possess greater amounts of PKD than normal basal keratinocytes. So as the authors wrote, the question remain: are the enhanced PKD1 levels in BCCs are simply a marker of their basal origin or does this elevated PKD1 contributes to the pathogenesis of BCCs.[11] Thus, another question is currently adequate, lack of PKD1 expression in SCCs despite increase expression of EGFR, is a consequence of its spinous layer origin, or is a consequence of PKD1 gene mutation(s) (silencing, methylation) as a result of gene alterations connected with the progression of precancer to cancer.[27,1,11]

As our analyzis shows, BCC do not express the classical and novel PKC isoforms—α, δ, η, ε (and ζ)[236], which could activate PKCμ/PKD1, upregulated in BCC. The lack of PKCα and PKCζ expression in BCC is also probably connected with leak inflammatory reaction in this cancer. Thus, the only PKC mediators of EGFR signaling in BCC are PKCι and PKD members, if PKD2 and PKD3 are expressed in BCC—there are no data. PKD1 is overexpressed in BCC: (1) increased expression of PKD1 is detected by Ristich *et al.*, using sc-935 antibody of Santa Cruz Biotech., which detects C-end of PKD1, but not pSer[738]/pSer[742] (pSer[777]/pSer[748] in mouse)-detected expression, not activity;[11] (2) the immunodetection showed nuclear staining—the kinase is active;[11] and (3) if the kinase is inactive, its silencing will lead to increased expression of metalloproteinases (MMPs) and EMT activation-cancer progression, BCC expressed relatively low levels of MMPs, in comparison with SCC[32] (hormone insensitive phenotype in breast and prostate cancer.[143,32,144] Thus, PKD1 is probably active in BCC, but the mechanisms connected with it activation are still unclear (there is data for PKC-independent PKD activation,[6,7], with exception of SRC and c-Abl-dependent PKD1 activation[6,7]), as well as the factors (signal pathways), connected with its increased expression. One possible mechanism connected with its upregulation is increased activity of NF-kB pathway (see PKD1 transcriptional regulation), activated in both BCC and SCC.[27] In regard of the new data concerning perpetuates *PRKD1* downregulation through beta-catenin/MYC/MAX protein complex ([25]—Figure 1; see also-protein kinase D1—transcriptional regulation), we can add that Bonifas *et al.* showed very high *c-MYC* expression in epidermis and at least 15-fold lower in BCC. In the same study, of eight BCC tested by RPA, all had near-complete loss of *c-MYC* mRNA as compared with that in HEK, epidermis, or whole skin ([145]; see protein kinase D1 in cancer). This could be one of the main mechanisms regulating expression of PKD1 in normal human keratinocytes, SCC and in BCC.

If PKD1 is not phosphorylated (activated) in activating loop, as a result of lack of classical PKCα and novel PKCs in BCC, it will be inactive and will not translocate into the nucleous, deactivating Snail, which will lead to early induction of EMT and tumor progression. Mutation in PKC genes in BCC are rare, but mutations in HH pathway, leading to high Gli1 activity, inducing expression of Snail (and PKCι, a positive feedbacksystem that can amplify Gli1/HH signaling independent of upstream inputs,[146]), inhibiting E-cadherin expression, and additionally activating β-catenin activity, stimulating cell proliferation, EMT, and tumor development.[27] Inactive PKD1 will lead to increase susceptibility of tumor development as a result of inability of phosphorylation of Ser11 of Snail, inhibiting EMT. [27] The data do not exist for other kinases, which could phosphorylate PKD1 in activation loop (authors' remark), except

UVB induced SRC and c-Abl-dependent PKD1 activation, which is PKC independent (see PKD1 activation)[6,7] and recent data showing that the kinase domain of PKD dimerizes in a concentration-dependent manner and autophosphorylates on a single residue in its activation loop upon DAG production.[14,13,21] Src family kinases (SFKs) play an important role in cancer proliferation, survival, motility, invasiveness, metastasis, and angiogenesis. Among the SFKs, c-Src and c-Yes are particularly overexpressed or hyper-activated in many human epithelial cancers. Western blotting and immunohistochemical staining showed that c-Src was expressed in all malignant skin tumors, but not in normal skin, while c-Yes was expressed in malignant melanoma (MM) and squamous cell carcinoma (SCC), but not in basal cell carcinoma (BCC) and normal skin.[147] Arun *et al.* have detected increased SRC, c-Abl-induced PKD1 activity in mouse keratinocytes after UVB exposure, the main pathogenetic cause for the development of BCC.[65,19,148,27] In addition, Montagner *et al.* unveil a cascade of events involving peroxisome proliferator-activated receptor (PPAR) β/δ and the oncogene Src, which promotes the development of ultraviolet (UV)-induced skin cancer in mice. UV-induced PPARβ/δ activity, which directly stimulated Src expression, increased Src kinase activity, and enhanced the EGFR/Erk1/2-signaling pathway, resulting in increased epithelial-to-mesenchymal transition (EMT) marker expression. Consistent with these observations, PPARβ/δ-null mice developed fewer and smaller skin tumors, and a PPARβ/δ antagonist prevented UV-dependent Src stimulation. Furthermore, the expression of PPARβ/δ positively correlated with the expression of SRC and EMT markers in human skin squamous cell carcinoma (SCC), and critically, linear models applied to several human epithelial cancers revealed an interaction between PPARβ/δ and SRC and TGFβ1 transcriptional levels. Taken together, these observations motivate the future evaluation of PPARβ/δ modulators to attenuate the development of several epithelial cancers.[149]

Since SRC and EGFR appear to cooperate to increase tumorigenicity, dual inhibition of SRC and EGFR has been proposed, such as in head and neck squamous cell carcinoma and colorectal cancer. However, SRC negatively regulates RAS by phosphorylation at Y32; therefore, SRC inhibition leads to increased RAS activity. In fact, EGF stimulation of SRC/YES/FYN triple knockout MEFs does not induce the phosphorylation of RAS as Y32, and as such exhibit increased RAS–RAF-1 interactions.[26]

In addition, during the process of terminal differentiation, the precise coordination between cell-cycle arrest and differentiation is required.[150] PKC*η*-mediated differentiation and growth arrest could be linked to activation of Fyn, a Sarc (SRC) kinase family member, and down modulation of EGFR-signaling pathways.[151,150] However, PKCη is not detected in basal cell epithelioma (Basal Cell Carcinoma).[150] CaSR (calcium-sensing receptor) regulates E-cadherin mediated cell–cell adhesion through the Rho/Fyn-dependent pathway. Inhibiting CaSR expression blocks the $[Ca^{2+}]_o$-induced activation of Rho and Fyn, the tyrosine phosphorylation of β-, γ-, and p120-catenin, the formation of E-cadherin/catenin complex, and activation of PI3K, connected with keratinocytes differentiation,[152] effect which could be also possibly observed as a result of inhibition of Fyn.

Although BCC are with basal origin, EGFR is expressed at a significantly higher level in SCC than in BCC.[153] Basal cell carcinoma (BCC) of the skin is a highly compact, non-metastatic epithelial tumor type that may arise from the aberrant propagation of epidermal or progenitor stem-cell (SC) populations. Increased expression of GLI1 is a common feature of BCC and is linked to the induction of epidermal SC (stem cells) markers in immortalized N/Tert-1 keratinocytes. Neill *et al.* demonstrated that GLI1 overexpression is linked to additional SC characteristics in N/Tert-1 cells including reduced epidermal growth factor receptor (EGFR) expression and compact colony formation that is associated with repressed extracellular signal-regulated kinase (ERK) activity. Colony formation and repressed ERK activity remain evident when EGFR is increased exogenously to the basal levels in GLI1 cells revealing that ERK is additionally inhibited downstream of the receptor. Exposure to epidermal growth factor (EGF) to increase ERK activity and promote migration negates GLI1 colony formation with cells displaying an elongated, fibroblast-like morphology. However, as determined by Snail messenger RNA and E-cadherin protein expression, this is not associated with epithelial–mesenchymal transition (EMT), and GLI1 actually represses induction of the EMT marker vimentin in EGF-stimulated cells. Instead, live cell imaging revealed that the elongated morphology of EGF/GLI1 keratinocytes stems from their being 'stretched' due to migrating cells displaying inefficient cell–cell detachment and impaired tail retraction. Taken together, these data suggest that GLI1 opposes EGFR signaling to maintain the epithelial phenotype.[154] However, Gli overexpression could be consider as a consequence of first or second mutation (first hypermethylation of 14-3-3σ). BCCs possess both PKD1 and Gli increased expression (see above) and ERK activity was predominantly negative in 13/14 BCCs (superficial/nodular).[154] However, this is not the right model for studying BCCs, since N/Tert-1 keratinocytes possess mutation in $p16^{INK4A}$, which is not typical for BCCs.[27,32]

In basal cell carcinoma (BCC) cells, where PKCι was revealed as an interacting partner to missing-in-metastasis (MIM), a scaffold protein necessary for Hedgehog signaling. PKCι was found to directly bind to and phosphorylate GLI1, the major transcription factor of the HH pathway, at residues within its zinc finger DNA-binding region. This trigger transcription of GLI1-dependent genes, among which is Snail,[133] one of the three transcriptional factors activating EMTransition (characterized with expression of mesenchymal cell markers). When these residues were

mutated to non-phosphorylatable alanines (S243A and T304A), there was decreased DNA binding detected by chromatin immunoprecipitation (ChIP) and thus PKCι would appear to phosphorylate GLI1 to potentiate transcription of HH signaling components. As PKCι is itself a GLI1 target gene, this sets up a positive feedback system that can amplify HH signaling independent of upstream inputs [smoothened (SMO) and suppressor of fused (SuFu) mutations] and lead to tumor growth.[146] The main function of Snail is inhibition of E-cadherin expression, liberation of active β-catenin, and EMT induction.[132,27]

**Protein kinase D1 in cancer**

Function of PKD1 in premalignant diseases (PMDs), epithelial-to-mesenchymal transition (EMT), Basocellular Carcinoma (BCC), and Spinocellular Carcinoma (SCC) was recently discussed from us in.[27,32] In brief, PKD1 phosphorylates Ser11 (S11) on transcription factor Snail, a master EMT regulator and repressor of E-cadherin expression, triggering nuclear export of Snail via 14-3-3σ binding,[156] inhibiting EMT in prostate cancer cells. Bastea *et al.* showed that PKD1-mediated phosphorylation of Snail at Ser11 reduces its binding affinity for its co-repressor Ajuba and leads to increased expression of E-cadherin. Phosphorylation of Ser11 by PKD1 also leads to the interaction between Snail and FBXO11. FBXO11 is an E3 ubiquitin ligase that targets Snail for ubiquitination and degradation by the proteasome machinery. The phosphorylation of Snail at Ser11 also generates a 14-3-3σ-binding motif and leads to 14-3-3σ-mediated nuclear export needed for proteasomal degradation. As a consequence of downregulation of Snail, E-cadherin is not repressed and EMT is not induced.[12] Snail S11 mutation causes acquisition of mesenchymal traits and expression of stem-cell markers.[156] Early mutations in 14-3-3σ (the 14-3-3 isoform responsible for Snail1 export, highly expressed in epithelium,[27] are detected in pancreatic cancer and in BCCs, connected also with early increased Snail1 activity, and EMT promotion (in pancreatic cancer phosphorylated Ser11 of Snail could not be bound and exported from thenucleous from 14-3-3σ, being transcriptionally active.[131,58] [,27] Concomitantly early high PKD1 expression is observed in these cancers.[57,27,11]

The data further support the hypothesis that PKD1 and E-cadherin regulate β-catenin activity in the same signaling pathway. PKD1 suppresses transcription factor Snail, a known E-cadherin repressor by inhibitory phosphorylation, induces expression of E-cadherin and upregulates β-catenin. The authors found that PKD1 activity influences the posttranslational modifications and transcription activity of β-catenin via T120 phosphorylation, which paradoxically decrease β-catenin transcription activity. Unphosphorylated-T120 β-catenin localized more to the nucleus compared to wild-type β-catenin.[25] Since the expression of Wnt target genes in C4-2/PKD1 [because C4-2 expresses low level of PKD1, a stable C4-2 cell line that expresses PKD1 was established (C4-2/PKD1)] cells is less than those in C4-2 cells (derived from LNCaP prostate cancer cells), the nuclear β-catenin in C4-2/PKD1 cells, which consists of phosphorylated T120 β-catenin, is less transcriptionally active, suggesting that β-catenin protein level alone is insufficient to count signaling activity.[121,157,131,156,12]

PKD1 interacts with β-catenin and phosphorylates also $Thr^{112}$.[12] $Thr^{112}$ is also a CK2 phosphorylation site, which may increase the binding between β-catenin and α-catenin and may promote β-catenin degradation, increasing Axin (scaffold proteins) binding. N-terminal residues $S^{29}$, $T^{102}$, and $T^{112}$ as CK2 phosphorylation sites *in vitro*. The A29/A102/A112 β-catenin mutant (substitution with alanine) is largely localized in the cytoplasm, and thus, it could be a target of the destruction complex. However, A29/A102/A112 β-catenin has enhanced (augmented) stability correlating with decreased affinity to Axin (scaffold proteins within a "destruction" complex—for proteasome-mediated proteolysis).[29,158] CK2 phosphorylation enhanced binding to GSK3β and α-catenin, and this appeared to favor adhesion. The major CK2 phosphorylation site in this domain is $Thr^{393}$, a solvent-accessible residue in a key hinge region of the molecule. Mutation of this single amino-acid reduces β-catenin phosphorylation, co-transcriptional activity, and stability. Thus, CK2 is a positive regulator of Wnt signaling through phosphorylation of β-catenin at $Thr^{393}$, leading to proteasome resistance and increased protein and co-transcriptional activity.[29] Matrix metalloproteinases (MMPs) are a family of more than 28 enzymes that were initially identified on the basis of their ability to cleave most elements of the extracellular matrix (ECM) but have subsequently been found to be upregulated in nearly every tumor type.[159] In normal tissue, MMPs are expressed at very low levels, with exception of MMP-10[114] and MMP-28,[160] but their production and activation is rapidly induced when active tissue remodeling is needed. Cell migration, keratinocyte hyperproliferation and angiogenesis are essential in both wound healing and tumor invasion. MMPs (metalloproteinases) have been associated with EMT in cancer progression by three distinct mechanisms: (a) elevated levels of MMPs in the tumor microenvironment can directly induce EMT in epithelial cells, (b) cancer cells that undergo EMT can produce more MMPs, facilitating cell invasion and metastasis, and (c) EMT can generate activated stromal like cells that drive cancer progression via further MMP production.[161] The most dramatic of these is MMP-dependent activation of the EMT program, seen in a variety of epithelial cell types, including kidney, ovary, lens, lung, and prostate, although MMP-induced EMT has been best characterized in mammary epithelial cells. Tumors that developed in the WAP-MMP-3 mice showed mesenchymal characteristics, and dissection of this process revealed that exposure of cultured mouse mammary epithelial cells to MMP-3 directly activates EMT. MMP-3 mediates these effects by stimulating increased expression of Rac1b, a constitutively activated splice variant of Rac1 found in breast and colorectal cancer cells, which in turn triggers EMT by increasing levels of cellular reactive oxygen species. While the process by which MMP-3 initiates these

effects has not been completely defined, MMP-3 has been shown to cleave E-cadherin, promoting dissolution of epithelial cells and releasing a bioactive fragment of E-cadherin that induces cell motility. [161]

PKD1 downregulate the expression of breast cancer cell MMPs, MMP-2, MMP-7, MMP-9, MMP-10, MMP-11, MMP- 13, MMP-14, and MMP-15, by so far unknown mechanisms[12] while upregulating expression of MMP-3, providing a potential mechanism for downregulated PKD1 mediation of the invasive phenotype.[162,27,32,160] For MMP-3 expression in SCC and BCC see [236].

Additionally, Baker J *et al.* show that both IL-1- and OSM-induced phosphorylation of protein kinase D (PKD) in human chondrocytes is strongly associated with signalling via the atypical PKCι isoform. Consequently, inhibiting PKD activation with a pan-PKD inhibitor significantly reduced the expression of MMP1/13. Specific gene silencing of the PKD isoforms revealed that only PKD3 (PRKD3) depletion mirrored the observed MMP repression, indicative of the pharmacological inhibitor specifically affecting only this isoform. PRKD3 silencing was also shown to reduce serine phosphorylation of signal transducer and activator of transcription 3 (STAT3) as well as phosphorylation of all three mitogen-activated protein kinase groups. This altered signalling following PRKD3 silencing led to a significant reduction in the expression of the activator protein-1 (AP-1) genes FOS and JUN, critical for the induction of many MMPs including MMP1/13. Furthermore, the AP-1 factor activating transcription factor 3 (ATF3) was also reduced concomitant with the observed reduction in MMP13 expression. Taken together, the authors highlight an important role for PKD3 in the pro-inflammatory signalling that promotes cartilage destruction.[77]

BCC is rarely metastatic cancer on contrary to SCC, but locally infiltrative and invasive, processes which also needs degradation of extracellular matrix. Recent research in this field identified MTA1 (metastasis-associated protein 1) as a novel substrate for PKD1, and show that PKD1-mediated phosphorylation of MTA1 triggers its polyubiquitination and proteasomal degradation. PKD1-mediated downregulation of MTA1 was accompanied by a significant suppression of prostate cancer progression and metastasis in physiologically relevant spontaneous tumor models. Accordingly, progression of human prostate tumors to increased invasiveness was also accompanied by decreased and increased levels of PKD1 and MTA1, respectively.[64] Moreover, the authors propose that this leads to the shuttling of PKD1/β-catenin complexes to E-cadherin, with the effect of stabilizing cell–cell contacts.[64]

A recent study identified, for the first time, a functional crosstalk between PKD2 and Aurora A kinase in cancer cells. Aurora A kinase (AURKA) is a master cell-cycle regulator that is often dysregulated in human cancers. Its overexpression has been associated with genome instability and oncogenic transformation. The data demonstrate that PKD2 is catalytically active during the $G_2$-M phases of the cell cycle, and inactivation or depletion of PKD2 causes delay in mitotic entry due to downregulation of Aurora A, an effect that can be rescued by overexpression of Aurora A. Moreover, PKD2 localizes in the centrosome with Aurora A by binding to γ-tubulin. Knockdown of PKD2 caused defects in centrosome separation, elongated $G_2$ phase, mitotic catastrophe, and eventually cell death via apoptosis. Mechanistically, PKD2 interferes with Fbxw7 function to protect Aurora A from ubiquitin- and proteasome-dependent degradation. Taken together, these results identify PKD as a cell-cycle checkpoint kinase that positively modulates $G_2$-M transition through Aurora A kinase in mammalian cells.[155]

While an inhibitory effect of PKD1 on cell migration and EMT has been the focus of multiple studies, little to nothing is known on the roles of PKD2 and PKD3 in the regulation of EMT. Indirect evidence indicates that PKD2 and PKD3 are potential positive regulators of EMT,[12] inducing MMPs expression.[37]

In addition, vitamin D signaling regulates cell proliferation and differentiation, and epidemiological data suggest that it functions as a cancer chemopreventive agent.[103,163-165,110,94,10,119,127,166] A large prospective study associated vitamin D sufficiency with reduced total cancer incidence and mortality, particularly in digestive cancers head and neck squamous cell carcinoma (HNSCC), esophageal, pancreatic, stomach, and colorectal cancers and leukemias. VDR gene polymorphisms also correlate with protection against different malignancies, including HNSCC.[31]

The role of VDR in EMT process and skin cancer was recently reviewed from us in: [103,163-165,110,94,10,119,127,166]

Vitamin D signaling can suppress expression of genes regulated by c-MYC, a transcription factor that controls epidermal differentiation and cell proliferation, whose activity is frequently elevated in cancer. The authors show through cell and animal-based studies and mathematical modeling that hormonal 1,25-dihydroxyvitamin D (1,25D) and the vitamin D receptor (VDR) profoundly alter, through multiple mechanisms, the balance in function of c-MYC and its antagonist the transcriptional repressor MAD1/MXD1. 1,25D inhibited transcription of c-MYC-regulated genes *in vitro*, and topical 1,25D suppressed expression of c-MYC and its target setd8 in mouse skin, whereas MXD1 levels increased. 1,25D inhibited MYC gene expression and accelerated its protein turnover. In contrast, it enhanced MXD1 expression and stability, dramatically altering ratios of DNA-bound c-MYC and MXD1. MXD1 levels correlate with epithelial differentiation, and MXD1's expression in SCCs is associated with the capacity of cells to differentiate. Remarkably, F-box protein FBW7, an E3-ubiquitin ligase, controlled stability of both arms of the c-MYC/MXD1 push–pull network, and FBW7 ablation attenuated 1,25D regulation of c-MYC and MXD1 turnover. In addition, c-MYC expression increased upon VDR knockdown, an effect

abrogated by ablation of MYC regulator β-catenin (vitamin D signaling suppresses β-catenin function). c-MYC levels were widely elevated in vdr−/− mice, including in intestinal epithelium, where hyperproliferation has been reported, and in skin epithelia, where phenotypes of VDR-deficient mice and those overexpressing epidermal c-MYC are similar. Thus, 1,25D and the VDR regulate the c-MYC/MXD1 network to suppress c- MYC function, providing a molecular basis for cancer preventive actions of vitamin D.[31]

In regard of the new data concerning perpetuates *PRKD1* down regulation through beta-catenin/MYC/MAX protein complex,[25] we can add that Bonifas *et al.* showed very high *c-MYC* expression in epidermis and at least 15-fold lower in BCC. In the same study, of eight BCC tested by RPA all had near-complete loss of *c-MYC* mRNA as compared with that in HEK, epidermis, or whole skin.[145] On contrary increased MYC gene copy number was observed in 7 of 23 CKA [classic keratoacanthoma (benign variant of invasive SCC)] and 17 of 19 SCC.[167] In another study, the c-Myc protein was overexpressed in 80% of the tumors of the oral cavity from the South Indian population.[168] Increased PKD1 expression was detected in BCC, but not detected in SCC.[11] The above-mentioned *PRKD1* regulation could be one possible mechanism leading to high PKD1 expression in BCC in comparison with Actinic keratoses (premalignant disease for SCC; transformation into BCC is 10%[148] and SCC possessing high C-Myc expression (activity).[145,27,169,170] Although the results of Bonifas *et al.* are absolutely obvious, according to other authors c-Myc is not downregulated in BCC.[169,170]

However, pancreatic cancers have shown increased c-Myc expression and direct or indirect MYC inhibitors is a novel concept in targeted treatment of PDAC.[171,28] Notably, MXD1 is not expressed (protein detection) in pancreas—islets of langerhans; breast—glandular cells, myoepithelial cells; liver—hepatocytes; cerebral cortex—neuropil; and soft tissues—peripheral nerve, chondrocytes (https ://www. prote inatl as.org/ENSG0 00000 59728 -MXD1/tissu e/primary+data).

As it was mentioned above, the treatment of C4-2 prostate cancer cells (more invasive derivative of LNCaP cells and with comparatively lower expression of *PRKD1*) with MYC inhibitor increased the transcriptional expression of *PRKD1* confirming the negative regulatory role of MYC on *PRKD1* expression. MYC inhibitor treatment also decreased the proliferation rate, migration and invasive ability of the cells,[25] but did not induce differentiation.[172]

Additionally, as it was mentioned above the impact of specific co-mutations in epidermal growth factor receptor (EGFR)-mutated lung adenocarcinoma is unclear. Tissues from 147 consecutive patients with resected EGFR-mutated lung adenocarcinomas treated at Sun Yat-Sen University Cancer Center were analyzed by next-generation sequencing (NGS). It was estimated that TP53 and protein kinase D (PKD) mutations were the two most frequently observed co-mutations in this cohort. Dual PKD/EGFR and TP53/EGFR mutations were found in 39 (27%) and 72 patients (49%), respectively, with dual TP53/EGFR mutations more commonly observed in male patients (P = 0.021). Both TP53 (hazard ratio [HR] 2.08, 95% confidence interval [CI] 1.23-3.54, P = 0.007) and PKD co-mutations (HR 1.72, 95% CI 1.01-2.93, P = 0.044) were associated with shorter disease-free survival (DFS), but not overall survival (OS), in univariate analysis. In multivariate analysis, patients harboring PKD/TP53 co-mutations had shorter DFS compared with $PKD^-/TP53^-$ cases (HR 2.49, 95% CI 1.15-5.37, P = 0.02). In a subgroup of never-smokers, TP53 co-mutations were associated with significantly worse OS (HR 50.11, 95% CI 2.39-1049.83, P = 0.012).[201]

**Protein kinase D2 and D3 in different cancer types**

Similar to the intricate roles played by many kinases, PKD1 has a complex relationship with respect to cancer development. PKD1 has been shown to be downregulated in prostate cancer, breast cancer, gastric cancer, and colon cancer. However, the overexpression of PKD1 has been shown to play a role in the development of pancreatic cancer and skin cancers (basocellular carcinoma—BCC). Hotspot-activating mutations of *PRKD1 (*p.Glu710Asp amino-acid substitution in the activation loop) have recently been identified in polymorphous low-grade adenocarcinomas of salivary glands and likely constitute oncogenic drivers in these tumors.[27,173,174] Therefore, the consequence of upregulation or downregulation of PKD1 in cancer development is dependent on the tissue type. Because PKD1 functions as a critical kinase that integrates extracellular signals into intracellular processes by modulating a multitude of signaling pathways, the regulation of PKD1 levels and/or activity through pharmacological or genetic intervention might aid in cancer treatment.[4]

Reviews of PKD1 as a potential new target for cancer therapy were published recently:[27,1,173,37,4]. Data are summarized in Table 1.

Advanced was made in the field of breast cancer. Breast tumors can be divided into different molecular subtypes: (1) the Luminal A and B subtypes, expressing high levels of estrogen and/or progesterone receptors; (2) the HER2 + subtype, overexpressing the human epidermal growth factor receptor 2 (HER2) protein; and (3) the triple-negative breast cancers (TNBC), expressing none of the hormone receptors and showing no HER2 amplification and/or overexpression. TNBC and HER2 + cancers are the most aggressive tumors with the highest metastatic potential. The poor prognosis of TNBC also results from the lack of treatment options for these patients, who cannot benefit from either hormone or HER2-targeted therapies. Although hormone-sensitive tumors can be treated with endocrine drugs, resistance is observed in about 40% of advanced-stage cases. Thus, it remains very important to identify new targets and associated biomarkers for breast cancer therapy.[173]

In recent cohort, Spasojevic *et al.* observed that PKD1 is downregulated in primary breast tumors as

compared to normal breast tissue (data not shown) and the authors also showed that high *PRKD1* mRNA levels are predictive of a poorer prognosis in both the entire cohort and the TNBC subgroup. The authors analyzed *PRKD1* mRNA levels in 527 primary breast tumors. They found that high *PRKD1* mRNA levels were significantly and independently associated with a low metastasis-free survival in the whole breast cancer population and in the triple-negative breast cancer (TNBC) subtype specifically. High *PRKD1* mRNA levels were also associated with a low overall survival in TNBC. Pharmacological inhibition and siRNA-mediated depletion of PKD1 reduced colony formation in MDA-MB-436 TNBC cells. PKD1 inhibition also reduced tumor growth *in vivo* in a TNBC PDX model. Together, these results establish PKD1 as a poor prognostic factor and a potential therapeutic target in TNBC. [173]

PKD1 is highly expressed in ductal epithelial cells of the normal breast, while its expression is downregulated in highly invasive breast cancers, by PKD1 gene promoter methylation. On the other hand, highly invasive breast cancers are characterized by increased expression of PKD3. Loss of PKD1 and upregulation of PKD3 in invasive breast cancer suggest that in this malignancy, PKD1 functions as a tumor suppressor, while PKD3 functions as an oncoprotein.[12] PKD1 blocks cell motility, whereas PKD2 and PKD3 seem to drive this event.[37] Unlike PKD1 and PKD3, the expression pattern of PKD2 remains relatively unchanged during breast cancer progression. However, evidence from multiple studies indicates that it also supports breast cancer development by promoting cell migration, proliferation, and multi-drug resistance.[38,12,37]

Borges *et al.* reported that high *PRKD3* expression is a poor prognostic factor in ER-breast tumors. However, Spasojevic *et al.* were not able to reach the same conclusions in their series of tumors. The biological role of PKD1 in breast cancer is still unclear, but the authors have previously demonstrated that it can drive estrogen independence in ER + BC cells. Most interestingly, high *PRKD1* expression is a poor prognostic factor in ER + tamoxifen-treated breast tumors, suggesting that PKD1 participates to endocrine therapy resistance in the clinics. In their last study, they found that *PRKD1* expression is associated with a poor prognosis specifically in TNBC, i.e., in another hormone-independent BC subtype. Together, these data indicate that PKD1 is likely to play a specific role in estrogen-independent breast tumors.[173]

Compared to consistent tumor-suppressive functions of PKD1 in breast cancer, how PKD2/3 functions in breast cancer are not fully understood. In the current study, Liu Y *et al.* found that PKD2 and PKD3 but not PKD1 were preferentially overexpressed in breast cancer and involved in regulating cell proliferation and metastasis. Integrated phosphoproteome, transcriptome, and interactome showed that PKD2 was associated with multiple cancer-related pathways, including adherent junction, regulation of actin cytoskeleton, and cell cycle-related pathways. ELAVL1 was identified as a common hub-node in networks of PKD2/3-regulated phosphoproteins and genes. Silencing ELAVL1 inhibited breast cancer growth *in vitro* and *in vivo*. Direct interaction between ELAVL1 and PKD2 or PKD3 was demonstrated. Suppression of PKD2 led to ELAVL1 translocation from the cytoplasm to the nucleus without significant affecting ELAVL1 expression. Taken together, Liu Y *et al.* characterized the oncogenic functions of PKD2/3 in breast cancer and their association with cancer-related pathways, which shed lights on the oncogenic roles and mechanisms of PKDs in breast cancer.[175]

According Liu Y *et al.* PRKD2 and PRKD3 were preferentially expressed in breast cancers. Immunohistochemistry confirmed the overexpression of PRKD2 and PRKD3 in TNBC. CRT0066101, which inhibited the activity of PRKDs, dramatically inhibited proliferation, increased apoptosis and the G1-phase population of TNBC cells *in vitro*, and reduced breast tumor volume *in vivo,* using also small interfering RNAs against PRKD2 and PRKD3 (siPRKD2 and siPRKD3). PRKD inhibitor CRT0066101 exhibits anti-TNBC effects via modulating a phosphor-signaling network and inhibiting the phosphorylation of many cancer-driving factors, including p-MYC(T58/S62), p-MAPK1/3(T202/Y204), p-AKT(S473), p-YAP(S127), and p-CDC2(T14), providing insight into the important roles as well as the molecular mechanism of CRT0066101 as an effective drug for TNBC.[176]

The studies not only highlight the significance of PKD signaling in prostate cancer progression, but also strongly suggest isoform-specific functions and contrasting roles for PKD1 and PKD3 in prostate cancer cells.[86] Common prostate cancer cell lines also display differential expression of the PKD isoforms. The LNCaP cell line, an androgen-sensitive and less metastatic cell line, expresses PKD1 and PKD2 only, while DU145 and PC3 cells, both androgen-insensitive and highly metastatic prostate tumor cells, express primarily PKD3, with moderate expression of PKD2 and no detectable PKD1.[1] Studies have shown that PKD1 and PKD3 expression levels are elevated in human prostate carcinoma tissues compared to normal prostate epithelial tissue, and advanced-stage tumors were found to have increased PKD3 nuclear accumulation. In contrast, androgen-independent tumors showed reduced PKD1 expression.[144,1]

Authors demonstrated a positive role for the PKD3 isoformin prostate cancer cell proliferation and survival. In PC3 cells, reduction of PKD3 levels using siRNA caused potent inhibition of cell proliferation. Analysis of a potential mechanism revealed that PKD3 activity stimulated prolonged activation of Akt and ERK1/2. This regulation of Akt and ERK1/2 may account for the effects on proliferation and also may affect other steps in prostate cancer progression. Akt, commonly found to be hyperactive in prostate cancer due to a phosphatase and tensin homolog (PTEN)-null phenotype, has been implicated in angiogenesis and metastasis in addition to its fundamental roles in survival and proliferation. La Valle *et al.* also found

that the inhibition of PKD activity using a novel chemical inhibitor of PKD not only reduced proliferation in LNCaP, DU145, and PC3 prostate cancer cells, but also significantly slowed migration and invasion of PC3 and DU145 cells. These studies not only highlight the significance of PKD signaling in prostate cancer progression, but also strongly suggest isoform-specific functions and contrasting roles for PKD1 and PKD3 in prostate cancer cells.[1]

Mast cells are being increasingly recognized as critical components in the tumor microenvironment. Protein Kinase D (PKD) is essential for the progression of prostate cancer, but its role in prostate cancer microenvironment remains poorly understood. PKD2/3 contributed to mast cells recruitment and tumor angiogenesis in the prostate cancer microenvironment. Clinical data showed that increased activation of PKD at $Ser^{744/748}$ in prostate cancer was correlated with mast cell infiltration and microvascular density. PKD2/3 silencing of prostate cancer cells markedly decreased mast cells migration and tube formation of HUVEC cells. Moreover, PKD2/3 depletion not only reduced SCF, CCL5 and CCL11 expression in prostate cancer cells but also inhibited angiogenic factors in MCs. Conversely, exogenous SCF, CCL5 and CCL11 reversed the effect on MCs migration inhibited by PKD2/3 silencing. Mechanistically, PKD2/3 interacted with Erk1/2 and activated Erk1/2 or NF-κB signaling pathway, leading to AP-1 or NF-κB binding to the promoter of scf, ccl5 and ccl11. Finally, PKD-specific inhibitor significantly reduced tumor volume and tumor growth in mice bearing RM-1 prostate cancer cells, which was attributed to attenuation of mast cell recruitment and tumor angiogenesis.[177] Data are summarized in Table 2.

**Discussion:**

According to Rashel *et al.*, the hyperplastic and inflammatory responces to topical phorbol ester were significantly supressed in PKD1-deficient mice suggesting involvement of PKD1 in tumor promotion. Consistently when subjected to two-stage chemical skin carcinogenesis protocol, PKD1 deficient mice were resistant to papilloma formation when compared to the control littermates.[87] Papilloma formation is mostly connected with increased c-Myc expression, which down-regulates p21.[27,178,115] These facts rise several questions 1. Increased PKD1 expression down-regulates E-cadherin/ β-catenin (stabilization of β-catenin and binding to E-cadherin on the cell membrane) and ERK1/2 signal pathways, but upregulate NF-κB and PI3K, which induce c-Myc. Which is the molecular event which determines decreased papilloma formation in PKD1 knock-down mice? According to Chiou *et al.* in DMBA/TPA-mediated tumors, the levels of both c-Myc and p21 increase (increased protein levels of PKD1 also), and both of them are inhibited by peracetylated (-)-epigallocatechin-3-gallate (AcEGCG).[136] Sustained activation of c-Myc is suficient to induced papillomatosis together with angiogenesis changes that resemble hyperplastic actinic keratosis, a commonly observed human precancerous epithelial lesion. All these premalignant changes spontaniously regress upon deactivation of c-MycER.[179] c-Myc deficient epidermis is resistant to Ras-mediated DMBA/TPA-induced tumorigenesis (7,12-dimethylbenz(a)-anthracene (DMBA)-initiated and 12-O-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumorigenesis in ICR mice). This is mechanistically link to $p21^{Cip1}$, which is induced in tumors by the activated Ras-ERK pathway, but repressed by c-Myc. Acute elimination of c-Myc in established tumors leads to upregulation of $p21^{Cip1}$, and epidermis lacking both $p21^{Cip1}$ and c-Myc reacquires normal sensitivity to DMBA/TPA-induced tumorigenesis. This identifies c-Myc mediated repression of $p21^{Cip1}$ as a key step for Ras driven epidermal tumoroginesis;[178] 2. If PKD1 deficiant mice were resistant to papillloma formation when compared to control littermates,[87] does it mean that PKD1 influences c-Myc or $p21^{Cip1}$ expression?, our unpublished results have showed that PKD1 do not influences expression and/or phosphorylatiom ($Ser^{62}$/$Thr^{58}$) of c-Myc in normal human keratinocytes; and 3. What is the molecular mechanism connected with c-Myc down-regulation in BCC (c-Myc is not down-regulated in pancreatic cancer, although possesing also early increased PKD1 expression)?[171,27,180,79]

SCC typically exibits a broad spectrum of progressively advanced malignanceis, ranging from premalignant actinic keratosis (AK) (precursor lesion) to squamous cell carcinoma in situ (SCCIS) to invasive cSCC and finally metastatic cSCC. The primary risk factor for AK is chronic UV exposure, and the estimated rate for an individual lesion to progress to cSCC is between 0.025% and 16% per year. Histologically, AKs are characterized by dysplasia of the keratinocytes of the basal layer often accompanied by parakeratosis and thinning of the granular layer. This localized epidermal atypia reflects a partial disruption of differentiation is associated with cSCC.[181] Genetically, Aks and cSCC are associated with amplification and activating mutations of the Ras oncogene indicate that 11% of cSCCs harbor activating Ras mutations (6% HRAS, 3% NRAS, 2% KRAS, n=371 cases). In cSCCs, Ras is frequently activated, but with low frequency of mutation.[181,27] The frequency of Ras mutations in BCC ranges from 10% to 30% (50%).[27,60] Expression of p16 and c-Myc could not be used for diffrentiation of AK from SCC, because of the similar staining, although increased expression of both p16 and c-Myc during the progression of skin from AK to in situ cSCC and to invasive SCC was observed.[170,163,182,183,184] Thus at least expression of c-Myc and PTCH1 inactivating mutations (90% of sporadic BCC)[27] could be used for differentiation of AK from BCC[148]. There are no data for the expression of PKD1 in AK and in other inflammatory skin diseases (PMDs). In regard of AK, results of Arun *et al.*[65] and Bollag *et al.*[19], which detected increased PKD1 activity after UVB irradiation, tempts us to speculate increased PKD1 activity/ expression in these lesions.

Beyond the four main genetic lesions in KRAS, CDKN2A (encoding $p16^{INK4A}$), TP53, and SMAD4

(TGFβ signaling), genetic heterogeneity, a hallmark of PDAC (Pancreatic ductal adenocarcinoma), is a crucial contributor towards the failure of therapies in the clinic. PDAC is usually driven by the mutated KRAS oncogene, is estimated to become the second leading cause of cancer-related deaths in the United States between the year 2020 and 2030. The 5-year survival rates remain dismal at about 8%. The squamous subtype of the disease, which overlaps with the quasi-mesenchymal subtype defined by Collisson *et al.* is characterized by an activated MYC pathway and the poorest outcome. These observations, moreover, reinforce MYC being a relevant driver in PDAC (and colorectal cancer - CRC) and argue that MYC targeting strategies are needed.[171,28,202] As the result of Nickkholgh *et al.* shows MYC inhibitor treatment decreased the proliferation rate, migration and invasive ability of the pancreatic cancer cells, increased the transcriptional expression of *PRKD1* (the gene of PKD1).[25]

It is thought that oncogenic KRas is an initial event leading to pancreatic cancer. Oncogenic KRas upregulates the epidermal growth factor receptor (EGFR) and its ligands TGFα and EGF, which leads to additional activation of wild-type KRas, and activity of both pathways is needed for pancreatic tumorigenesis. Major downstream signaling cascades activated by active KRas in pancreatic cancer are the PI3-K/PDK1/Akt (known also as PKB) and Raf/MEK1/2/ERK1/2 (extracellular signal-regulated kinase) pathways.[57] Approximately 95% of all pancreatic ductal adenocarcinoma (PDAC) express either somatic activating mutations of Kras or show increased epidermal growthfactor receptor (EGFR) signaling (activated by TGFα), both of which leads to activation of PKD1.[55,56]

In response to such signaling, acinar cells undergo acinar-to-ductal metaplasia (ADM). During ADM cells, downregulate expression of PKD3 and upregulate expression of PKD1, whereas PKD2 expression remains unchanged. In addition to increased expression, PKD1 activity is also elevated in presence of a mutant Kras, or after EGFR-mediated activation of endogenous wild-type Kras. As a result of such signaling, PKD1 expression and activity can be detected in regions of ADM, PanIN1, and PanIN2 pre-neoplastic lesions, while the two other PKD isoforms are not involved in these processes. Questions remaining are (1) how PKD1 expression is upregulated by both, mutant Kras and EGFR signaling? And (2) how both pathways can mediate activation of PKD1? Since PKD1 activity downstream of Kras was determined by measuring nPKC-mediated activating phosphorylations, an involvement of the novel PKCs PKCε and/or PKCδ is most likely.[57]

Doppler *et al.* and Eiseler *et al.* have shown that PKD1 regulates ADM by activating the Notch pathway, which previously had been established as a driver of acinar cell reprogramming. On one hand, active PKD1 downregulated the expression of known suppressors of Notch (e.g, Cbl, Sel1l). On the other hand, active PKD1 also upregulated the expression of Notch target genes (e.g., Hes-1, Hey-1), molecules that are involved in Notch signaling (e.g., MAP2K7), stem cell markers (e.g., CD44), as well as proteinases, including Adam10, Adam17, and MMP7, that mediate Notch activation by S2 cleavage. PKD1 has been shown to activate nuclear factor κ-B (NF-κB); and NFκB and Notch both cooperate in some signaling pathways (reviews:[55,56] A crosstalk between Notch and canonical NF-κB-signaling pathways is needed for progression of pancreatic cancer and PKD1 is a key enzyme linking KRas to Notch and NF-κB. Therefore, PKD1 could be a promising new target to treat pancreatitis, prevent precancerous lesions and tumor formation, but also progression of tumors.[22,57,59]

In normal squamous mucosa, strong membranous pattern of PKD1 staining as well as difused or granular cytoplasmic staining of PKD1 were observed, which is in contrast to the weak and diffused cytoplasmic staining of PKD1 in HNSCC tumor tissues.[140] In BCC PKD1 showed nuclear staining.[11] One possible explanation is that as a result of 14-3-3σ mutations, PKD1 can not be export into the cytosol and stay active into the nucleus. Early 14-3-3σ mutations are also detected in pancreatic cancer and BCC, leading to early Snail nuclear (transcriptional) activity, E-cadherin repression, and EMT promotion.[131,58,27,130] According to Lodygin and Hermeking there is over expression of 14-3-3σ in pancreatic cancer, associated with promoter hypomethylation, and downregulation in breast (in more than 90%), cervical, prostate, ovarian bladder and hepatocellular carcinoma, and in BCC, as a result of promoter CpG hypermethylation, mutations of the gene are not detected.[185,130] Although some tumors, such as ovarian tumors, oral squamous cell carcinomas, and pancreatic ductal adenocarcinomas, among others, show upregulation of the protein.[185] In breast cancer progression, the frequency of hypermethylation gradually increases upon transition from atypical hyperplasia to invasive breast carcinoma. Moreover, hypermethylation of *14-3-4*σ was detected in adjacent histologically normal breast epithelium, while CpG methylation in breast epithelial cells was never observed in individuals without evidence of breast cancer. It has been proposed that hypermethylation of *14-3-4*σ is an early event during breast cancer formation that precedes any morphological change in tissue architecture or cell shape. Lodygin *et al.* made a similar observation in tissues containing basal cell carcinoma (BCC) of the skin. Morphologically benign epidermis, adjacent to BCC, showed the presence of CpG methylation, whereas the epidermis derived from a distant location was free of *14-3-3*σ methylation. An inverse correlation between *p53* mutations and loss of *14-3-3*σ expression through promoter methylation has been reported for oral SCC.[130]

In human breast cancer, the regulation of 14-3-3σ may involve two mechanisms: ubiquitin-mediated proteolysis by Efp and downregulation by hypermethylation. However, the inactivation of 14-3-3σ is probably achieved mainly by hypermethylation. Silencing Efp in the MCF-7 breast cancer cell line resulted in increased expression of 14-3-3 σ. The Efp-

positive human breast cancers were more frequently 14-3-3σ-negative (60.5% *vs.* 39.5%). Hypermethylation of 14-3-3σ was common (64.9%) and had an inverse association with 14-3-3σ positivity ($p = 0.072$). Positive 14-3-3σ expression was significantly correlated with poor prognosis: disease-free survival ($p = 0.008$) and disease-specific survival ($p = 0.009$). Interestingly, 14-3-3σ turned out to be a very significant poor prognostic indicator, which is in contrast to its previously known function as a tumor suppressor, suggesting a different role of 14-3-3σ in breast cancer.[165]

Ling *et al.* demonstrated that loss of the conditional 14-3-3σ allele results in accelerated HER2/ERBB2-driven mammary tumorigenesis and metastasis. This study underscores the role of 14-3-3σ as a potent tumor suppressor in ERBB2-driven tumor initiation and progression.[186]

Because there is discrepancy in the scientific literature concerning expression of 14-3-3σ in pancreatic cancer, and in our previous analysis, we have made comparison between alterations (mutations) in BCC and pancreatic cancer (both with early downregulated 14-3-3σ and high PKD1 expression), we decide to search additional data, concerning this question.

14-3-3σ contributes to the development of prostate adenocarcinoma. 14-3-3σ expression is significantly decreased during the progression of normal prostatic epithelium to prostatic intraepithelial neoplasia and invasive cancer.[187] 14-3-3σ protein is downregulated in human prostate cancer cell lines, LNCaP, PC3, and DU145 compared with normal prostate epithelial cells. Among prostate pathological specimens, > 95% of benign hyperplasia samples show significant and diffuse immunostaining of 14-3-3σ in the cytoplasm, whereas < 20% of carcinoma samples show positive staining. In terms of mechanisms for the downregulation of 14-3-3σ in prostate cancer cells, hypermethylation of the gene promoter plays a causal role in LNCaP cells.[188] Low 14-3-3σ levels are found in many types of human tumors due to allelic loss and/or promoter hypermethylation.[185]

14-3-3σ expression was determined in 5 pancreatic cancer cell lines. In research of Guweidhi *et al.* 14-3-σ mRNA levels were analyzed using QRT–PCR and 54-fold increased in pancreatic adenocarcinoma in comparison with normal pancreatic samples was detected.[189] Neupane *et al.* found also that the cancer cells in 7 PDAC samples expressed high levels of 14-3-3σ mRNA by quantitative PCR when compared with normal pancreatic duct cells. 14-3-σ protein levels were high in BxPC3, COLO-357, and T3M4 cells, intermediate in ASPC-1 cells, and low in PANC-1 cells. Most cell lines released detectable amount of 14-3-3σ into conditioned medium. Overexpression of 14-3-σ in PANC-1 cells led to resistance to cisplatinum-induced apoptosis, increased basal migration, and increased invasion in response to epidermal growth factor and insulin-like growth factor-I. By contrast, short hairpin RNA-mediated knockdown of endogenous 14-3-3σ in T3M4 cells did not alter migration but led to enhanced cisplatinum sensitivity, increased invasiveness in response to epidermal growth factor, and decreased invasiveness in response to insulin-like growth factor-I.[190] The increased expression of 14-3-3σ may contribute to the failure in treatment of pancreatic cancers.[191]

Zhaomin *et al.* found that 14-3-3σ protein level was increased significantly in about 71% (17 of 24) of human pancreatic cancer tissues and that the 14-3-3σ protein level in cancers correlated with lymph node metastasis and poor prognosis. Furthermore, the author demonstrated that overexpression of 14-3-3σ in a pancreatic cancer cell line caused resistance to γ-irradiation as well as anticancer drugs by causing resistance to treatment-induced apoptosis and G2/M arrest. Thus, 14-3-3σ may serve as a prognosis marker predicting survival of pancreatic cancer patients and guide the clinical treatment of these patients.[191]

In another study, Hustinh *et al.* examined two-hundred forty-four (82%) primary infiltrating adenocarcinomas of the pancreas demonstrated positive expression of the 14-3-3σ, 45 (15%)showed weak immunolabeling, and 9 (3%) were negative. Ezrin (ERM, Radixin, Moesin) has been identified as being important in the metastatic behavior of pancreatic and other cancers. 201 (68%) showed positive immunolabeling of the ERM proteins, 75 (25%) demonstrated weak expression and 20 (7%) no expression. A similar proportion of ampullary cancers showed 14-3-3 σ and ERM protein expression. Expression of 14-3-3σ and ERM protein was more likely in poorly differentiated cancers ($p = 0.00005$), and their expression was associated with poor survival in univariate analysis ($p = 0.09$). By multivariate analysis, patients whose cancers expressed 14-3-3σ, but not ERM tended to have a poorer prognosis (Hazard ratio 1.4; 0.9–2.2, $p = 0.14$). Aberrant expression of 14-3-3σ may contribute to the outcome of patients with pancreatic ductal adenocarcinoma.[164]

Together with $p21^{WAF1}$, 14-3-3σ belongs to a subset of p53 targets which mediate cycle arrest, whereas other p53 target genes mediate programmed cell death. p53 was found to directly activate the transcription of 14-3-3σ after DNA damage in colorectal cancer cells. ΔNp63α, an isoform of the p53 homolog p63, which lacks a *trans*-activation domain, was shown to bind to the p53-responsive element in the 14-3-3σ promoter, but, in contrast to p53, represses transcription of 14-3-3σ. Interestingly, 14-3-3σ was shown to participate in the nuclear export of ΔNp63α protein and thereby promote its proteosomal degradation after DNA damage in squamous cell carcinoma of the head and neck. BRCA1 was shown to affect G2/M progression by inducing expression of 14-3-3σ.[130] In addition, 14-3-3σ was shown to bind to G1-specific CDKs (cyclin dependent kinases), such as CDK2 and CDK4, the interaction between 14-3-3σ and CDKs is not dependent on ligand phosphorylation, and might, therefore, depend on the availability. [130]

Experimental inactivation of 14-3-3σ in primary keratinocytes by an anti-sense approach allows keratinocytes to evade senescence and become

immortal without additional genetic alterations. How the loss of 14-3-3σ contributes to immortalization is not clear. However, it is possible that the epigenetic silencing of 14-3-3σ in keratinocytes may contribute to BCC formation by inactivation of senescence pathways which normally limit the proliferative capacity. Moreover, 14-3-3σ-deficient cells show increased genomic instability, characterized by loss of telomeric repeat sequences, chromosome end-to-end fusions and non-reciprocal translocations, characteristic for aneuploidy. Breast cancer cells with hypermethylation of 14-3-3σ display increased genomic instability. [130]

In our previous analysis we could not succeed in finding data connected with 14-3-3σ expression in SCC.[27] Currently, the immunoreactive 14-3-3σ protein was detected mainly in the cytoplasm of differentiated squamous cells of oral SCC lesions as well as adjacent nonmalignant squamous mucosa. Immunoreactivity for 14-3-3σ was observed in 93% of SCC lesions (27 of 29), including HPV-negative cases. No significant association was observed between 14-3-3σ expression and clinicopathologic parameters. A statistically significant correlation was found between 14-3-3σ protein expression and the Ki-67 labeling index. 14-3-3σ expression was correlated inversely with HPV-16 E6.[192]

In another study no mutations were detected in the coding sequence of 14-3-3σ in 20 oral carcinomas, and there was loss of heterozygosity in only 7 of 40 informative cases. In contrast to the absence of genetic change, aberrant methylation within 14-3-3σ was detected in 32 of 92 (34.78%) squamous cell carcinomas and in 3 of 6 (50%) oral dysplasias and was associated with reduced or absent expression at both mRNA and protein levels. Methylation was not detected in matched, normal epithelial tissue controls. Carcinomas in which 14-3-3σ was methylated were significantly more likely to lack DNA sequences from human papillomavirus and to have coincident methylation of $p16^{INK4a}$ than cases that expressed 14-3-3σ. Methylation was detected in SCC, both wild type and mutant for p53, but was more commonly detected in cancers with wild-type p53. These results implicate coincident epigenetic abrogation of function in both σ and $p16^{INK4a}$ in a subset of SCCs of the oral cavity.[193] HPV(+) SCC have shown also lack of hypermethylation in $p16^{INK4a}$ (CDKN2A) and mutations in Ras, and only 3% amplification of c-Myc and 3% mutations in p53.[27,49,194]

In esophageal squamous cell carcinoma, the positive expression rates of cytoplasmic and nuclear 14-3-3σ were 61.7% and 41.9%, respectively. There was no correlation between 14-3-3σ and p53 expressions. Positive expression of nuclear 14-3-3σ was significantly correlated with depth of invasion, stage, lymphatic invasion, and poor prognosis.[107]

To assess whether the potentially high-risk (HR) human papillomavirus (HPV)-related upregulation of 14-3-3σ (stratifin) has implications in the outcome of HPV infections or cervical intraepithelial neoplasia (CIN) lesions, cervical biopsy specimens from 225 women in the Latin American Screening Study were analyzed for 14-3-3σ expression using immunohistochemical analysis. Expression of 14-3-3σ increased in parallel with the lesion grade. Upregulation was also significantly related to HR-HPV detection ($p = 0.004$; odds ratio 2.71; 95% confidence interval 1.37–5.35) and showed a linear relationship to HR-HPV loads ($p = 0.003$). 14-3-3σ expression was of no value in predicting the outcomes (incident, persistent, clearance) of HR-HPV infections or incident CIN1+ and CIN2+. 14-3-3σ is not inactivated in cervical carcinoma and CIN but is upregulated on transition from CIN2 to CIN3. Its normal functions in controlling G1/S and G2/M checkpoints are being bypassed by HR-HPV.[195]

The expression and methylation status of 14-3-3σ in human salivary gland adenoid cystic carcinoma (ACC) and mucoepidermoid carcinoma (MEC). Immunohistochemical analysis revealed that the positive expression rate of 14-3-3σ in ACC (one out of 14) was markedly lower than that in MEC (ten out of 10). Since most of the ACCs carried the wild-type p53 protein, downregulation of 14-3-3σ in ACC may not be due to the dysfunction of p53 pathway.[196]

In addition, 14-3-3σ-deficient cells show increased genomic instability, characterized by loss of telomeric repeat sequences, chromosome end-to-end fusions and non-reciprocal translocations. Breast cancer cells with hypermethylation of 14-3-3σ display increased genomic instability. Furthermore, RNAi-mediated downregulation of 14-3-3σ protein expression in prostate carcinoma cells was shown to compromise the stability of a DNA damage induced G2 arrest and promote polyploidisation (Lodygin *et al.*, unpublished results). Taken together, these results strongly argue for an important role of 14-3-3σ in the maintenance of genomic integrity after DNA damage and suggest that the epigenetic silencing of this gene in cancer cells may contribute to the chromosomal instability.[130]

In addition to its role in proliferation, there is increasing evidence that one important consequence of deregulated c-myc expression is the induction of genomic instability. c-Myc seems to favor gene amplification and gene rearrangements as well as karyotypic instability leading to numerical and structural chromosomal aberrations). The most intriguing features of c-Myc-induced alterations are their reversibility. Using inducible systems, genomic instability proved to be transient upon a single induction of c-Myc *in vitro* and *in vivo*. Continued c-Myc activation, on the other hand, was described to be accompanied by numerical and structural chromosomal changes such as extra-chromosomal elements and chromosomal breakage but also centromere-telomere fusions. Furthermore, in the absence of p53 the c-Myc effect seemed to be augmented and aberrant centrosome duplication was discussed as a potential mechanism.[197]

c-Myc may additionally contribute to genomic instability through a telomere organization-dependent mechanism. While in normal cells, the telomeres are present as non-overlapping territories, tumor cells (*in*

*vitro* and *in vivo*) with deregulated c-Myc as well as HaCaT cells constitutively expressing the c-myc oncogene, showed aggregation of telomeres in a high percentage of cells. Since these telomeric aggregates (TAs) could be detected in interphase nuclei as well as in mitotic figures and the percentage of apoptotic cells was not significantly increased as compared with the parental cells, their presence throughout the cell cycle is likely to contribute to unequal segregation of the chromosomes during mitosis, i.e., numerical aberrations. In addition, TAs also likely force the chromosomes to alter their location, thereby providing an explanation for how the different chromosomes come into such close vicinity that they are able to exchange chromosomal material and give rise to the in part very complex multi-chromosomaltranslocations characteristically seen in skin carcinoma cells, [197] aneuploidy especially seen in BCC.

The PKs genes are found to be disrupted throughout the genome, and in some positions distinct from those previously reported: PKCα is at 17q, PKCβ at 16p12, PKCγ at 19q13.4, PKCδ at 3p.21.2, PKCε at 2p21, PKCζ at 1p36.3, PKCη at 14q22-23, PKCθ at 10p15 and PKCι at 3q26[54] — Figure 1], and PKD1 at 14q12, PKD2 at 19q13.32, PKD3 at 2p22.2.

For PKCι, an additional fluorescence in situ hybridization (FISH) signal mapped on Xq21.3 revealed a pseudogene (derived by retrotransposition). PKCγ, ζ (in keratinocytes) and θ are found in map to the most distal parts of their chromosomes (PKCγ at 19q13.4, PKCζ at 1p36.3, and PKCθ at 10p15,[54] and we will add PKD2-19q13.32 before PKCγ) (PKCγ ans PKCθ are not expresssed in human keratinocytes), suggesting that there might be a telomeric position effect modifying these genes expression throughout the replicative lifespan of human cancer. However, there is no experimental evidence on this at present,[54] although that the rate of detected PKC mutations in BCC is low and we can not find data for their promoter hypermethylation in BCC, for SCC.[140]

**Conclusions:**

Protein kinase Ds (PKDs) are implicated in the regulation of a remarkable array of fundamental biological processes, including signal transduction, Golgi organization, regulating the fusion of vesicles from the *trans*-Golgi network (TGN), plasma membrane directed transport, insulin secretion and survival of pancreatic β-cells, cell-survival apoptosis, proliferation, differentiation, and migration.[7] PKD is also implicated as a mediator in stress and disease states, including human hypertrophic cardiomyopathy, the most common cause of sudden cardiac death in the young, Bcr-Abl-induced NF-κB activation in human myeloid leukemia, in oxidative stress responses (including UVB), immune regulation, inflammation—pancreatitis, diabetes, angiogenesis, and cancer.[5,7,4]

PKD1 posses proproliferative and antidifferentiative function in human and mouse keratinocytes,[19,198,10,11], prodifferentive function in hTert (N/Tert-1 or N-hTERT) keratinocytes[94,69] (and in osteoblasts[69]), participates also in wound-healing process in mouse epidermis.[86,87] Loss of PKD2 enhanced human keratinocytes (KC) proliferative potential, while loss of PKD3 resulted in a progressive proliferation defect, loss of clonogenicity and diminished tissue regenerative ability. This proliferation defect was correlated with upregulation of CDK4/6 inhibitor $p15^{INK4B}$ and induction of a p53-independent G1 cell-cycle arrest. Simultaneous silencing of PKD isoforms resulted in a more pronounced proliferation defect consistent with a predominant role for PKD3 in proliferating KCs.[82] There is no data concerning regulation of the PKD1, 2, and/or 3 kinase expression in skin.

According Ryvkin *et al.* epidermal tissue regenerated from PKD2-deficient KCs (sh-PKD2) showed a significant increase in epidermal thickness, although all differentiated layers of epidermis including granular and cornified layers were present. Immunostaining of tissue sections verified normal distribution of markers of early (INV) and late (FIL) epidermal differentiation; however, p63 staining was extended to upper spinous layers. Their findings indicated that PKD3 silencing in NHKCs coincide with a marked reduction in p63, a major regulator of proliferation and differentiation in epidermis. Similar to p63, PKD3 is predominantly expressed in the proliferative compartment of epidermis and is downregulated in differentiated KCs.[82] Additionally, ΔNp63α, implicated as an oncogene, is upregulated by activated Akt. Knockdown of ΔNp63α led to increases in PTEN levels and loss of activated Akt, while overexpression of ΔNp63α decreased PTEN levels and elevated active Akt, upregulating cyclin D1 in keratinocytes.[85] *In vivo* nuclear PTEN was absent in the proliferative basal layer of the epidermis where ΔNp63α expression is highest. This balance is disrupted in non-melanoma skin cancers through increased ΔNp63a levels, and could enhance proliferation and subsequent neoplastic development.[83]

In skin pathology, PKD1 is with increased expression in psoriatic lesions and BCC (basocellular carcinoma) and not detected in SCC.[11] In the multistage mouse skin carcinogenesis model, expression of PKD1 and CD34+ [cutaneous cancer stem-cell (CSC) marker] are increased with increased expression of p53, p21, c-Myc, cyclin B, p-CDK1 and Cdc25A and inhibited activation of extracellular signal-regulated kinase 1/2 (ERK1/2), increased nuclear factor-kappaB (NF-κB), cyclic adenosine 3′,5′-monophosphate-responsive element-binding protein (CREB) and CCAAT-enhancer-binding protein (C/EBPs) activation by increased phosphorylation of c-Jun-N-terminal kinase 1/2 (JNK1/2), p38 and phosphatidylinositol 3-kinase (PI3K)/Akt and by increased downstream target gene expression, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), ornithine decarboxylase (ODC) and vascular endothelial growth factor (VEGF) (in papillomas). Pretreatment with AcEGCG [peracetylated (−) epigallocatechin-3-gallate] before TPA treatment can be more effective than EGCG in reducing DMBA/TPA-induced tumor incidence and multiplicity, inhibiting the observed effects and acting as a chemopreventive agent.[136]

The hyperplastic and inflammatory responses in PKD1-deficient mice to topical phorbol ester were significantly suppressed suggesting involvement of PKD1 in tumor promotion (and inflammation). Consistently, when subjected to two-stage chemical skin carcinogenesis protocol, this mice were resistant to papilloma formation when compared to control littermates.[87] There are no other data for PKD1 participation in inflammatory skin process and expression in other premalignant skin diseases, with exception of psoriasis.[11]

Recently, hotspot-activating mutation in PKD1, resulting in an p.Glu710Asp amino-acid substitution, was detected in 73% of salivary Polymorphous low-grade adenocarcinoma (PLGA), associated with metastasis-free survival. The p.Glu710Asp (E710D) mutation, which lies within the catalytic loop of the PRKD1 kinase domain, led to significantly increased kinase activity compared with wild-type PRKD1 in an in vitro kinase assay (suggesting that this mutation activates PRKD1 (activating mutation). Interestingly, forced expression of the E710D mutant promoted proliferation and altered the glandular architecture of epithelial cells *in vitro*, further suggesting that this mutation confers a growth advantage to cells, but expression of the mutant protein reduced cellular migration (reduces invasion). Consistent with these findings, the presence of the *PRKD1* hotspot mutation was significantly associated with metastasis-free survival. The author's findings indicate that *PRKD1* mutations may have diagnostic and prognostic utility and distinguish indolent PLGA from more aggressive salivary gland tumors.[174]

High *PRKD1* mRNA expression as a single marker (HR 2.00, 95% CI 1.28–3.14, Wald's $p = 0.002$) and positive lymph node status (HR 4.00, 95% CI 2.22–7.37, Wald's $p$ 0.001) independently predicted for unfavorable diseasefree survival (DFS), clinicopathological factors required to accurately identify patients at high risk for recurrence in operable laryngeal cancer. According to authors, it seems worthy to prospectively validate *PRKD1* expression as a laryngeal cancer prognostic marker, for routine clinical applications. Patients that had undergone total laryngectomy had decreased risk for relapse (HR 0.55, 95% CI 0.31–0.95, Wald's $p = 0.036$), as compared to all other surgical approaches.[199] Its increased expression is connected with late phases of malignant melanoma, associated with high metastatic potential.[200] PKD1 participates in the pathology of inflammatory skin diseases and skin oncogenesis, but the mechanisms of regulation of its expression and action in pathological process in skin are still unsufficient.

TP53 and PKD mutations were the two most frequently observed co-mutations in resected EGFR-mutated lung adenocarcinoma. Both mutations were associated with poorer prognoses in affected patients. TP53 and protein kinase D (PKD) mutations were the two most frequently observed co-mutations in this cohort. Dual PKD/EGFR and TP53/EGFR mutations were found in 39 (27%) and 72 patients (49%), respectively, with dual TP53/EGFR mutations more commonly observed in male patients (P = 0.021). Both TP53 (hazard ratio [HR] 2.08, 95% confidence interval [CI] 1.23-3.54, P = 0.007) and PKD co-mutations (HR 1.72, 95% CI 1.01-2.93, P = 0.044) were associated with shorter disease-free survival (DFS), but not overall survival (OS), in univariate analysis. In multivariate analysis, patients harboring PKD/TP53 co-mutations had shorter DFS compared with $PKD^-/TP53^-$ cases (HR 2.49, 95% CI 1.15-5.37, P = 0.02). In a subgroup of never-smokers, TP53 co-mutations were associated with significantly worse OS (HR 50.11, 95% CI 2.39-1049.83, P = 0.012).[201]

PKD1 is upregulated in BCCs (basocellular carcinoma) and pancreatic cancer.[22,57,58,1,4] The mechanisms regulating PKD1 expression are merely studied in pancreatic and prostate cancer cells. It was recently demonstrated that KRas-induced activation of the canonical NF-κB pathway is one mechanism of how *PRKD1* expression is increased and identify the binding sites for NF-κB in the *PRKD1* promoter in pancreatic cancer.[22] Androgen deprivation gradually upregulated PKD1 protein expression. Androgen Receptor (AR) was required for the transcriptional repression of PKD1 gene expression caused by androgen stimulation in androgen-sensitive prostate cancer cells. However, the androgen response elements (AREs—two) in PKD1 promoter did not play an active role in regulating PKD1 transcription in response to androgen stimulation. The AR/NCOA1 complex stimulates migration of prostate cancer cells through suppression of *PRKD1*. Furthermore, *PRKD1* was negatively regulated by AR and *PRKD1* knockdown could significantly enhance the migratory potential of the two cell lines tested.[23,24] Novel auto-repressive loop that perpetuates *PRKD1* down regulation through β-catenin/ MYC/MAX protein complex, leading to increased nuclear β-catenin in prostate cancer was reported.[25]

Determining the interacting signaling pathways and direct downstream effectors of the PKD isoforms in regulating growth and differentiation of human epidermis is of great interest for future studies and will have therapeutic value in treating skin hyperproliferative disorders and cancer.[82] Targeting CD34 in conjunction with PKD1 inhibitors could become a viable option for the development of new and more effective multitarget strategies for preventing skin carcinogenesis.[136] Inhibiting or knocking-down PKD1 in cancer is connected with induction of EMT and more aggressive cancer phenotypes,[27] the hotspot-activating mutation in PKD1 in PLGA is associated with metastasis free survival.[174] From the other side, specific inhibition of protein kinase D1 (PKD1) allows the reversion of the metastatic phenotype in aggressive melanoma,[200] with second late increase of PKD1 expression,[27] induction of mesenchymal–epithelial transition (MET), at the site of metastases, reversed N- to E-cadherin switch, with a rapid cell shape modification from an elongated mesenchymal-like structure into a 'cuboidal' epithelial-like shape and a strong increase in cell–cell junctions.[200] It is, therefore, of high importance to make clear the role of PKD

isoforms in a relevant model to human skin to better understand which PKD to target and when, so that future translational strategies will be more effective.[82]

Zhang *et al.* data consistently showed that either knockdown or overexpression of PKD1 did not significantly alter the proliferation of HNSCC cells *in vitro*. However, interestingly, induction of PKD1 *in vivo* by Dox (doxycycline) provided a slight growth advantage to the HNSCC tumor xenografts and resulted in a significant increase in final tumor weight in Dox-induced *vs.* the non-induced tumors. This correlated to increased ERK1/2 and NF-κB signaling activity, and enhanced tumor-cell proliferation *in vivo*. Later, they demonstrated that in the presence of a mitogen (bombesin or GRP) that activates PKD, overexpression of PKD1 potentiated the mitogenic effects of bombesin in HNSCC, and depletion of endogenous PKD2, the predominant PKD isoform expressed in HNSCC cells, abolished such effect. At molecular level, overexpression of PKD1 promoted bombesin or GRP-induced ERK1/2 activation, while knockdown of PKD2 reduced EKR1/2 activation. It has been shown that the mitogenic effects of GRP are mediated by the activation of the MEK/EKR1/2 MAPK pathway through transactivating EGFR in HNSCC cells. The authors findings imply that PKD1 and PKD2 may contribute to the mitogenic effect of GRP and bombesin by facilitating the activation of ERK1/2. PKD2 mRNA was upregulated in seven out of ten tumors *vs.* normal in patient-paired HNSCC tissue specimens. Thus, it is possible that PKD2 plays a predominant role in the growth, survival, and motility of HNSCC cells, and these functions have compensated the loss of PKD1 in tumors. Transcript levels of PKD2 and PKD3 showed the opposite trend, i.e. increased PKD2 or PKD3 expression in tumor vs. normal tissue, authors data from PKD2-knockdown cells support this claim. Overexpression of PKD1 promoted the growth of HNSCC tumor xenografts.[140]

According recent researches, PKD1 is a potential therapeutic target for oral squamous carcinoma, knock-down of which inhibit the growth and promote the apoptosis of SCC-25 cells via downregulating Bcl-2 expression and downregulation of the expression of P-gp.[141] The study of Chen J *et al.*suggests that PKD1 may be a potential target for microenvironment-directed tumor biotherapy. They found that hypoxia not only induced the expression of HIF-1α, but also induced the expression and activation of PKD1 in the same SCC25 cells. Knock-down of PKD1 decreased the growth, as well as the expression of HIF-1α, glucose uptake, lactate production and glycolytic enzyme (GLUT-1 and LDHA) expression, as well as reduced the phosphorylation of p38 MAPK, while the percentage of apoptotic SCC25 cells was increased.[142]

In EMT (Epithelial to Mesenchymal Transition) cells PKD1 phosphorylates S11 of the transcriptional factor Snail, resulting in it export from nucleus to cytosol by 14-3-3σ, thus decreasing its transcriptional activity. Inhibition or knock-down of it will activate EMT.[27] In surrounding fibroblast inhibition of PKD1 have showed stimulatory effects on DNA synthesis.[237]

And finally in ECs (endothelial cells) - PKDs regulate both hypoxia-induced VEGF expression/secretion by the tumor cells and VEGF- stimulated angiogenesis, which are essential for the malignant progression of tumors. More and more studies show that PKD-1 signaling regulates the transcriptional expression of genes that are important in angiogenesis.[79] Thus the critical points in use of PKD1 inhibition in threatment of SCC are the EMT cells and in smaller axtent the surrounding fibroblasts.

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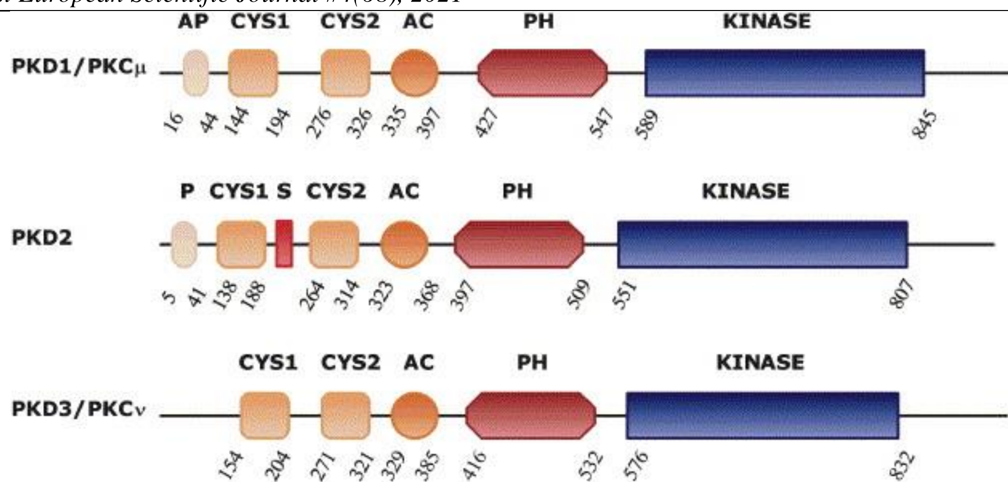



*Figure 1:*

Modular structure of PKD family members: PKD1/PKCμ, PKD2, PKD3/PKCν. AP alanine- and proline-rich domain, P proline- rich domain, S serine-rich domain, CYS cysteine-rich Zn-finger domain, AC acidic domain, PH pleckstrin homology domain, KINASE kinase catalytic domain (from Rykx et al. 20036 - with permission).

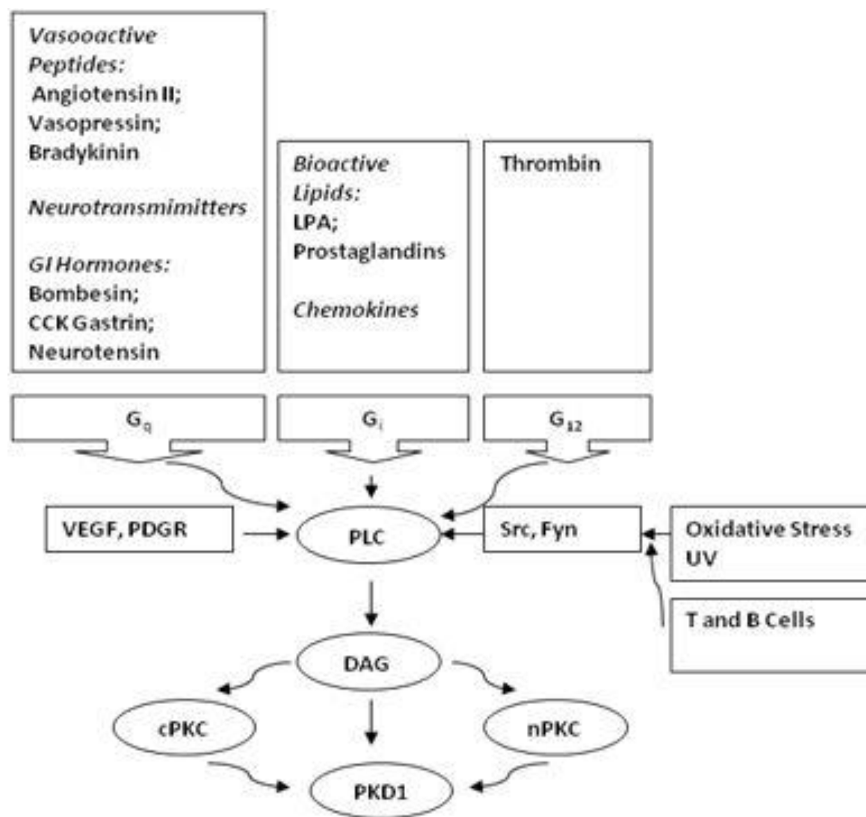



*Figure 2:*

PKDs activation by multiple stimuli: Hormones, growth factors, neurotransmitters, chemokines, bioactive lipids, proteases, and oxidative stress induce PLC-mediated hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP2) to produce DAG at the plasma membrane, which in turn mediates the translocation of inactive PKDs from the cytosol to that cellular compartment. DAG also recruits, and simultaneously activates, novel PKCs to the plasma membrane, which mediates trans-phosphorylation of PKD1 on Ser744 (in mouse PKD1). DAG and PKC-mediated trans-phosphorylation of PKD act synergistically to promote PKD catalytic activation and autophosphorylation on Ser748. The modular structure of PKD (mouse PKD1) is illustrated as an example of the PKD family. PKD1 is the most studied member of the family, and its knockout induces embryonic lethality. Further details are provided in the text. LPA lysophosphatidic acid; LPA stimulated the production of interleukin 8 (IL-8), a potent proinflammatory chemokine, and stimulated NF-κB activation, through PKD2 activation in NCM460 (human colonic epithelial) cells (modification of Rozengurt 20112).

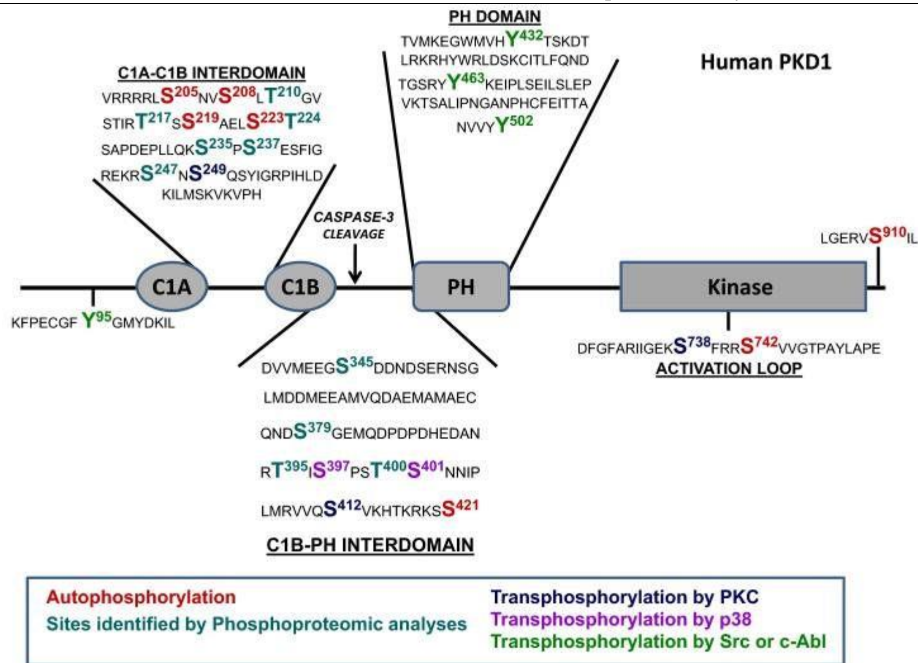



*Figure 3:*

Domain structure and regulatory phosphorylation sites in PKD1. C1A/C1B cysteine rich Zn-finger domains, Kinase domain. Numbering based upon the human PKD1 enzyme (from Steinberg 20127 - with permission).

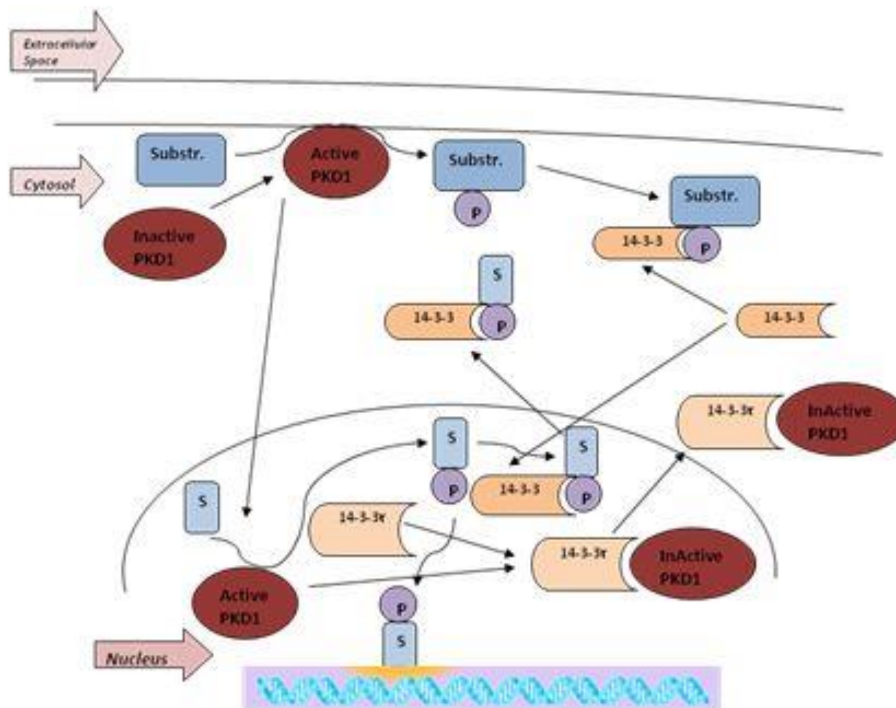
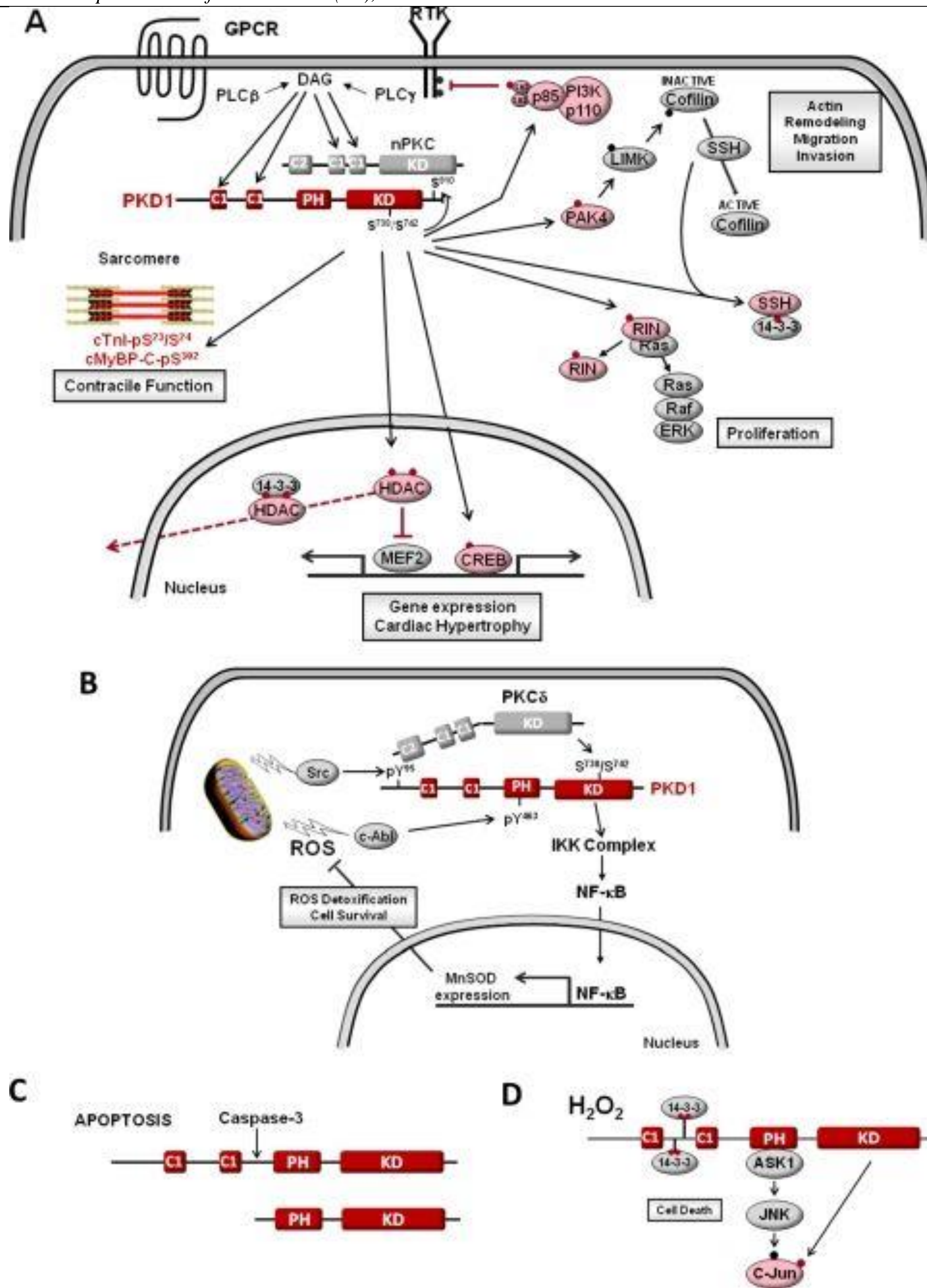



*Figure 4:*

Schematic representation of the mechanism by which PKD modulates intracellular localization of its substrates. In many cases, the phosphorylation of PKD substrates (Substr. (cytosolic) = S (nuclear)) induces binding of 14-3-3 proteins that sequester them to the cytosol, thereby preventing them from acting at the plasma membrane (e.g., RIN1, Par-1, DLC1) or at the nucleus (e.g., HDACs 5 and HDACs 7, Snail). An emerging theme is that PKD signaling regulates cell function by altering the subcellular localization of its substrates and its own (modification of Rozengurt 20112).




*Figire 5: PKD1 activation mechanisms.*

A: G proteincoupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) activate PKD1 via an allosteric mechanism involving lipid cofactors and phosphorylation by nPKC isoforms. PKD1 then phosphorylates a range of cellular substrates, including HDAC5, the sarcomeric proteins cTnI and cardiac myosinbinding protein C (cMyBP-C), CREB, the 27-kDa heat-shock protein (HSP27), p21 protein (Cdc42/Rac)-activated kinase 4 (PAK4), c-Jun, Bit1 (Bcl-2 inhibitor of transcription, a mitochondrial protein that induces caspase-independent apoptosis), the F-actin-binding protein cortactin, the cofilin phosphatase slingshot 1, RIN1 (a Ras effector protein that influences ERK and c-Abl pathways), and the p85 regulatory subunit of PI3K (which is inhibited—no longer binds to RTKs—when phosphorylated in the SH2 domain by PKD1); direct substrates of PKD1 are in pink. B and C depict alternative mechanism for PKD1 regulation by reactive oxygen species (ROS) or caspase-3 in the setting of oxidative stress or apoptosis (see Other PKD1 Activation Mechanisms) (from Steinberg 20127 - with permission).

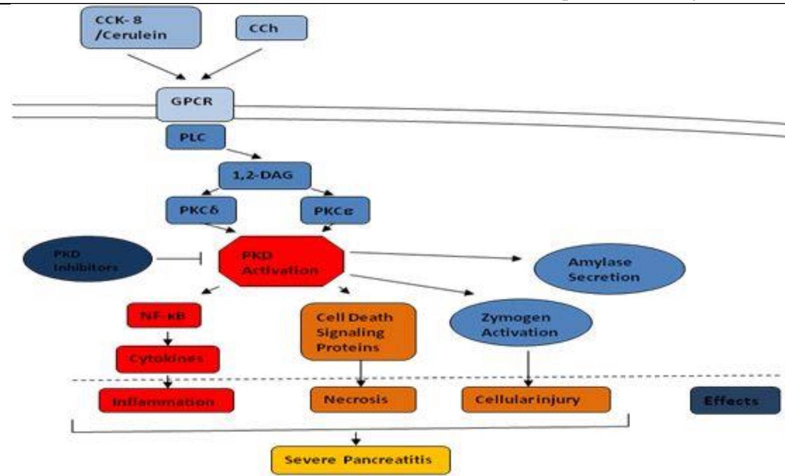



*Figure 6:*

A scheme summarizing the role of PKD in multiple pathological processes associated with pancreatitis. CCK/cerulein-induced PKD activation promotes necrosis (and decreases apoptosis) in pancreatitis by regulating multiple cell death signaling proteins. Active PKD also mediates NF-κB activation and inflammatory response, zymogen activation, and amylase secretion. These pathologic responses can be blocked when PKD is inhibited by specific PKD inhibitors, resulting in amelioration of the severity of pancreatitis. All these effects indicate that PKD may represent a potential therapeutic target in pancreatitis (modification of figure of Yuan and Pandol 201659).

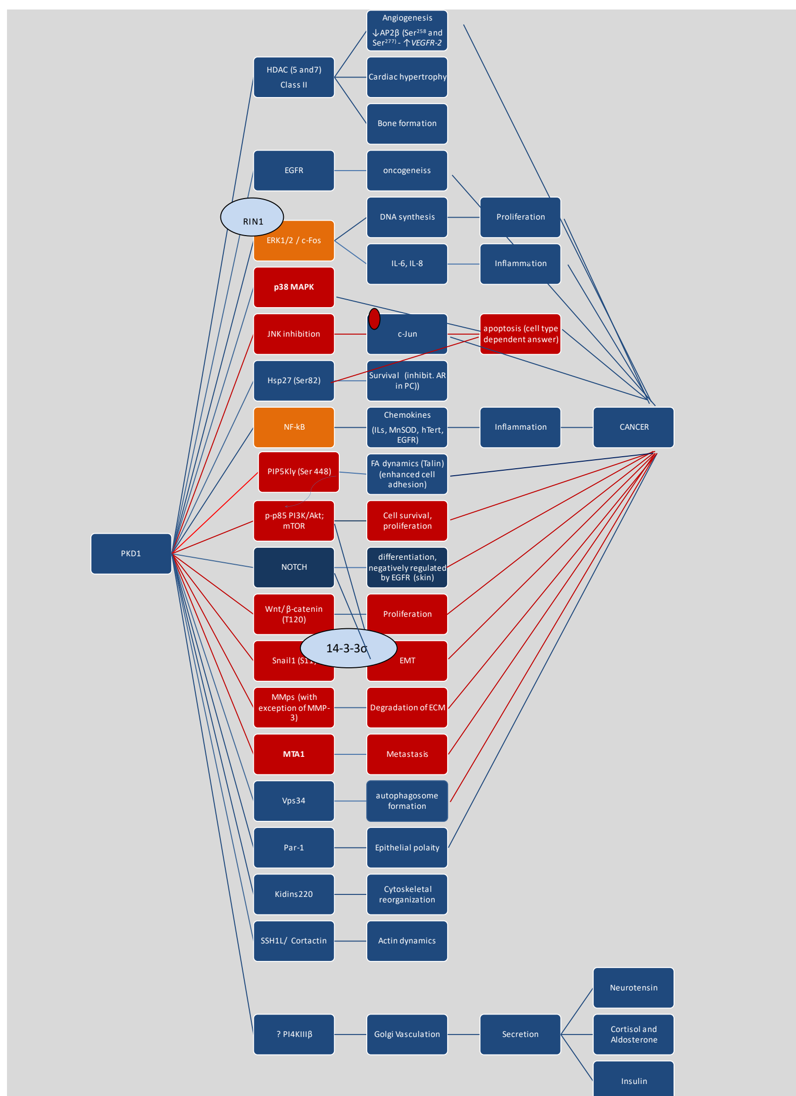



*Figure 7:*

Schematic representation of signaling pathways modulated by PKD1 in cancer. The deregulated expression of PKD1 results in the development of cancer. The schematic representation shows the pathways that activate PKD1, and the various downstream pathways and functions modulated by this kinase. The activation of various membrane receptors, such as GPCR and other growth factor receptors (GFR), leads to the activation of PKD1 by the PLC–PKC pathway through the formation of DAG. DAG modulates PKD1 function by binding and quickly recruiting it to the cell membrane for activation by PKCs. PKD1 can also be activated by the Golgi Gβγ–PKCε in the Golgi apparatus, by proteolytic cleavage by caspase 3, by oxidative stress resulting in PKD1 activation on mitochondrial surface through the action of SRC-Abl kinase and PKCδ, by UVB ray-activated SRC kinase, and by a PKC-independent self activation mechanism. Activated PKD1 is rapidly translocated from the membrane to the cytoplasm and eventually to the nucleus, where it regulates downstream pathways. Activated PKD1 also regulates the process of vesicle trafficking from the Golgi to the membrane, which eventually controls cell-surface proteins that are involved in cell adhesion, cell polarity, and motility. Depending on the cell type, PKD1 functions as either a tumor suppressor or an oncogene within the cell. PKD1 has been shown to inhibit cancer in the prostate, breast, and gastrointestinal tract. In blue pathways stimulated by PKD1 (prooncogenic), in red tumor suppressor functions of PKD1. PKD1 inhibits tumorigenesis by enhancing cell adhesion and inhibiting the function of proteins involved in cell migration, cell invasion, cell proliferation, and EMT. Some of the pathways that are modulated by PKD1 and may result in a prooncogenic role are as follows: PKD1-mediated activation of NF-κB also increases expression of epidermal growth factor receptor (EGFR) and its ligands transforming growth factor-alpha (TGFα) and epidermal growth factor (EGF).[57,68] PKD1 expression contributes to oncogenesis by enhancing angiogenesis by regulating the activity of HDAC5 and HDAC7 (see also Figure 5). Activated PKD1 enhances cell survival and proliferation by enhancing DNA synthesis and upregulating the function of ERK1/2 protein in the MAPK pathway, leading to the accumulation of c-Fos.[38,12,4] Downregulation of p38 MAPK signaling by PKD1 in response to hydrogen peroxide has been demonstrated to protect cells from apoptosis.[22] Activated PKD1 decreases apoptosis by suppressing the function of the JNK pathway, resulting in decreased c-Jun levels. Another target for ROS-activated PKD1 is the small heat-shock protein Hsp27, which is phosphorylated by PKD1 at S82. PKD1-phosphorylated Hsp27 can bind apoptosis signal-regulating kinase 1 to prevent JNK-induced apoptosis. Hsp27 also has been implicated in chemoresistance of several cancers.[22] Under oxidative stress conditions, PKD1 enhances cell survival through activation of the NF-kB pathway. The effects of PKD1 are mediated through direct phosphorylation of FA-localized phosphatidylinositol-4-phosphate 5-kinase typeI γ (PIP5KIγ) at serine residue 448. This phosphorylation occurs in response to Fibronectin-RhoA signaling and leads to a decrease in PIP5KIγs' lipid kinase activity (decreased production of PIP2) and binding affinity for Talin. PKD1 phosphorylate the p85 regulatory subunit of PI3K (which is inhibited—do not bind RTKs—when it is phosphorylated in the SH2 domain by PKD1.[7] PKD1 regulates acinar-to-ductal metaplasia (ADM) by activating the Notch pathway.[22,57,58,55,56,59] PKD1 phosphorylates E-cadherin and β-catenin, thereby enhancing cell–cell adhesion. PKD1 helps to maintain cellular polarity by phosphorylating Par-1 polarity-associated kinase and thus enhancing its cytoplasmic sequestration by 14-3-3 protein. Activated PKD1 also helps to establish cell polarity by positively regulating the TGN carriers to the basolateral membrane. Activated PKD1 can also inhibit the transcriptional activity of β-catenin and AR, resulting in reduced cell proliferation. It also inhibits EMT by regulating the activity (inhibition) of Snail transcription factor. PKD1 negatively regulates cell invasion by influencing the levels of MMPs through the modulation of HDACs. Reverse correlation between PKD1 and metastasis-associated protein 1 (MTA1) expression in samples of human prostate, colon, and breast cancers, in which PKD1 expression decreased and MTA1 expression increased, with progressed tumor grade or stage.[164] In addition, the tumor suppressor death-associated protein kinase phosphorylates and activates PKD1 in response to oxidative damage. Such signaling induces autophagy, due to PKD1-mediated phosphorylation of Vps34, which increases its lipid kinase activity and autophagosome formation.[22] It also negatively regulates actin remodeling and thus cell motility through the phosphorylation of SSH-1L and cortactin. The active, *trans*-Golgi network (TGN)-associated PKD1 activates its downstream targe PI4KIIIβ to trigger protein secretion to cell surface.[8] The upregulation of PKD1 has been linked to the development of pancreatic and skin cancer (BCC), and it may also be involved in prostate cancer (modification of Rozengurt 2011; [2] text from Sundram 2011; Durand *et al.* 2016; Durand *et al.* 2016[38,12,4]).

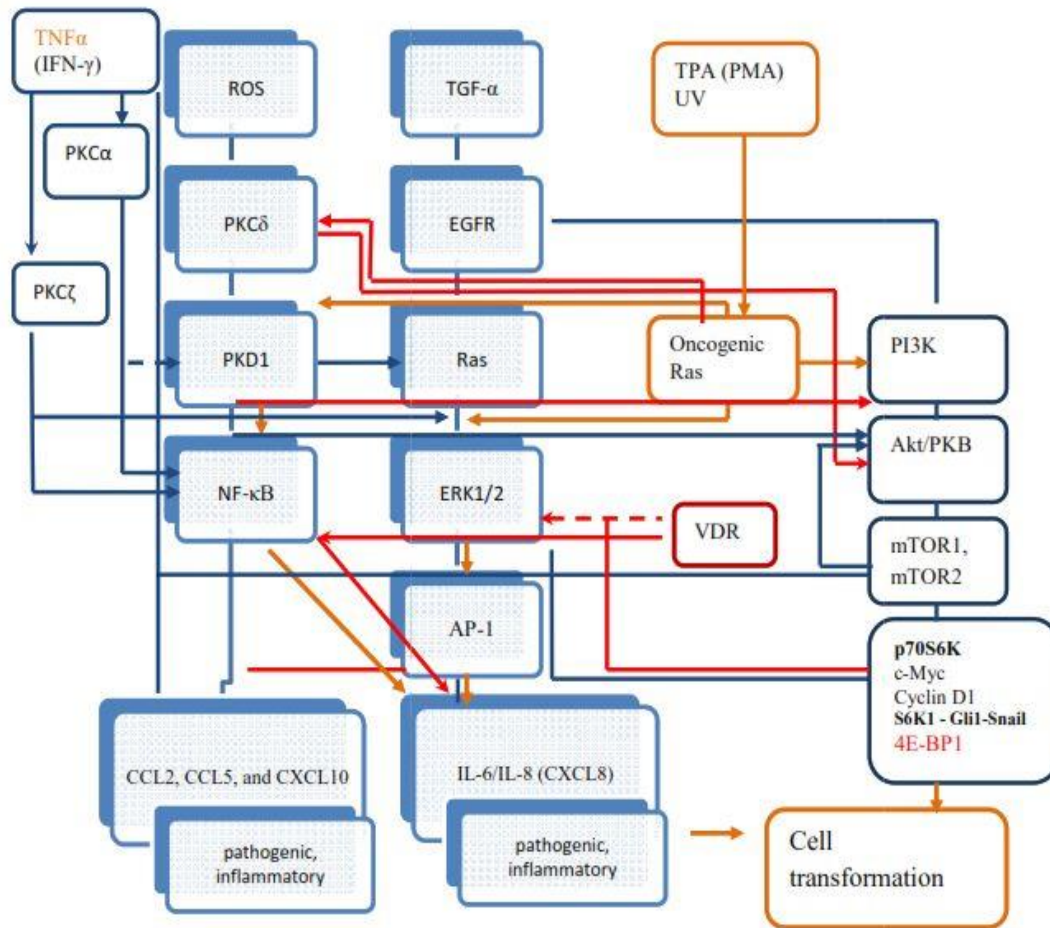



*Figure 8:*

Schematic representation of signaling pathways in inflammatory skin diseases: a common feature of chronic inflammatory skin disorders such as psoriasis, atopic dermatitis, and allergic contact dermatitis, is epidermal hyperplasia and thickening, a phenomenon attributed to leukocyte-derived cytokines such as tumor necrosis factor (TNF-α) and interferon (IFN-γ), which are potent inducers of EGF family growth factors and EGFR. In the course of T-cell-driven skin inflammatory diseases, activated Th1 lymphocytes infiltrating the dermis and the epidermis are the major source of IFN-γ and TNF-α. These cytokines initiate a program of increased keratinocyte expression of inflammatory mediators, including adhesion molecules, cytokines, and chemokines. In particular, prominent keratinocyte expression of CCL2 (monocytechemoattractant protein 1, MCP-1), CCL5 (RANTES), CXCL8 (IL-8), and CXCL10 (IFN-γ-induced protein of 10kd, IP-10) is a common finding in T-cell-mediated skin diseases, and mediates the recruitment of T cells and other leukocyte populations in the skin.[95,96] Only CXCL8 promoter activity could be impaired by ERK1/2 inhibition, confirming that ERK1/2- driven AP-1 *trans*-activation plays a relevant role in its transcription.[96] The rest of the explanations in the text;[96] It would appear that PKCα controls two distinct responses in keratinocytes, one related to growth and viability that is AP-1-dependent (induction of apoptosis) and the other related to chemotaxis and inflammation that appears to be independent of AP-1.[99] Because the proapoptotic activity of cutaneous PKCα is prevented by blocking the AP-1 pathway, the proximal affectors of apoptosis are likely to be AP-1-regulated genes. The expression of AP-1 factors is altered in neoplastic keratinocytes, and this could contribute to the differences in response to PKCα activation between normal and neoplastic keratinocytes. Elevated PKCα activity in neoplastic cells (lack in BCC) could also contribute to the inflammatory response in cutaneous neoplasms;[100] PKCζ is also critically located in the transduction pathway from TNF-α to activate NF-κB, the event crucial for the inflammatory process within the psoriasis lesions.[101] PKCζ is required for TNF-α signaling and nuclear factor-κB (NF-κB) activation.[101] PKCζ phosphorylates the IKKκ subunit *in vitro*, possibly through their direct interaction.[103] PKCζ activity is required for EGF-induced extracellular signal-regulated kinase (ERK) activation in both normal human adult epidermal keratinocytes and five of seven SCCHN (Spino cellular carcinoma head and neck) cell lines;[102] PKD1 is activated by oncogenic Ras expression and PKD1 promotes Ras OIS (Oncogene-induced senescence) by mediating inflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) via modulation of NF-κB activity. It was demonstrated that ROS-protein kinase Cδ (PKCδ)-PKD1 axis is essential for the establishment and maintenance of IL-6/IL8 induction. In addition, ablation of PKD1 causes the bypass of Ras OIS, and promotes cell

transformation and tumorigenesis in IMR90 human diploid fibroblasts.[66] Expression of oncogenic Ras, in response to various growth factors such as TGF*α* and EGF, or epidermal growth factor receptor (EGFR) activation can downregulate PKC-δ protein and steady-state mRNA levels in HaCaT cells, an immortalized, nontransformed human keratinocyte cell line. PKC-δ can also be negatively regulated by Ras at the protein levels by inhibitory tyrosine phosphorylation and/or by degradation in mouse keratinocytes. Ras oncogene or EGFR signaling are activated in a high percentage of human cancers, including cutaneous SCCs (up-expression in 90% of HNSCC,[156,27] and regulates transcription of many prosurvival and tumor suppressor genes via activation of a variety of transcription factors such as nuclear factor-κB, Ets, and activator protein-1. Thus, the mechanism of human PKC-δ downregulation/inactivation in human SCCs may involve regulation at multiple levels.[203,204] In response to TGFα and EGF—activates MEK–ERK, raf1, and ras, Src, ras, STAT-1 and -3; increases the levels of c-fos and Jun B while lowering those of c-jun and fra-1, activating Sp1 and AP-1 TFs.[151,205] PKCδ increases KLF4— stimulates Involucrin, p21[Cip1] expression, and upregulated VDR expression (prodifferentiative activity—PKC-independent, but AP-1 dependent).[205,206,166,207,208] UV (and other apoptotic stimuli) — generation of the constitutively active catalytic fragment, by caspase-3 cleavage of full-length PKCδ, collectively addressed as protein kinase M, PKM—triggers the redistribution and activation of Bax that can directly induce cytochrome c release (induction of apoptosis),[151,209] which is p53-independent.[204] PKCδ is a major isoform involved in both inhibiting Akt activity and enhancing UV-induced apoptosis in keratinocytes (de-phosphorylation of Akt on Ser[473]).[210] PI3Ks are activated by RTKs, such as EGFR, and the catalytic subunit phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to form phosphatidylinositol 3,4,5-triphosphate (PIP3). Explanations in the text.[46] Ras can also activate the PI3K signaling cascade[46,34] and RalGDS (Ras-like guanine nucleotide-dissociation stimulator).[34] Ziv *et al.* established that TNF activated ERK in an EGFR and Src-dependent and an EGFR and Src-independent modes. EGFR dependent activation resulted in the upregulation of the transcription factor, c-Fos, while the EGFR independent activation mode was of a shorter duration, did not affect c-Fos expression but induced IL-8 mRNA expression. Calcitriol, enhanced TNF-induced EGFR-Src-dependent ERK activation and tyrosine phosphorylation of the EGFR, but abolished the EGFR-Src independent ERK activation. These effects were mirrored by enhancement of c-Fos and inhibition of IL-8 induction by TNF. Treatment with calcitriol increased the rate of the de-phosphorylation of activated ERK, accounting for the inhibition of EGFR-Src independent ERK activation by TNF. It is possible that effects on the ERK cascade contribute to the effects of calcitriol and its synthetic analogs on cutaneous inflammation and keratinocyte proliferation.[119,120] Vitamin D receptor (VDR) physically interacts with IκB kinase β (IKKβ) to block NF-κB activation. 1,25$(OH)_2D_3$ rapidly attenuates TNFα-induced p65 nuclear translocation and NF-κB activity in a VDR-dependent manner.[125,127,166] VDR (Vitamin D Receptor) ligands inhibit expression of proinflammatory cytokines produced by T lymphocytes, such as IL-2, IFN-γ, IL-6 and IL-8 (trough NF-κB-binding sequence), which are responsible for the exacerbation of the skin inflammation. Apart from that, 1α,25$(OH)_2D_3$ enhances expression of anti-inflammatory cytokine, IL-10, within the psoriatic lesions, as well as the expression of its receptor in keratinocytes.[166,98] 1,25D and the VDR regulate the c-MYC/MXD1 network to suppress c- MYC function, providing a molecular basis for cancer preventive actions of vitamin D.[166,31] PKCδ[205,206,207] and VDR, induced human involucrin (hINV)[206] and p21[waf1/cip1 205] promoter activity, through specific binding elements of AP-1[207,31] and KLF4[205,206,208] promoter;[166] For PI3K/Akt/mTor pathway explanations in the text.

Table 1

**PKD1 expression and its roles in various cancers (modification of Sundram *et al.* 2011[4]).**

| No. | Cancer type | PKD1 status | Modulated signaling pathway | References |
|---|---|---|---|---|
| 1. | Breast cancer | Downregulated | MMPs | Eiseler T *et al.*, 2009[162] |
| | | | Actin remodeling and cell motility | Peterburs P *et al.*, 2009[211] |
| | | | EMT | Du C *et al.*, 2010[131] |
| 2. | Prostate cancer | Contentious | E-cadherin/β-catenin | Jaggi M *et al.*, 2003; 2005; 2008[144,212,213] |
| | | Downregulated | AR signaling | Mak P *et al.*, 2008[214] |
| | | | HSP27 | Hassan S *et al.*, 2009[215] |
| | | | EMT | Du C *et al.*, 2010[131] |
| | | | ERK/MAPK/MMPs | Biswas MH *et al.*, 2010[216] |
| 3. | Gastrointestinal cancer | Downregulated | β-catenin | Kim M *et al.*, 2008[217] |
| | | | Cox-2 | Jepperson T *et al.*, 2009[218] |

| | | | | Rodriguez Perez *et al.*, 2011[219] |
|---|---|---|---|---|
| 4. | Pancreatic cancer | Upregulated | Ras/Raf/MEK/ERK/RSK<br>HSP27<br>EGF/JNK<br>Apoptosis | Guha S *et al.*, 2002; 2003[220,221]<br>Yuan J *et al.*, 2008[222]<br>Kisfavli K *et al.*, 2010[223]<br>Trauzold A *et al.*, 2003[224] |
| 5. | Basocellular carcinoma (BCC) | Upregulated | dysregulation of Ras in 100% and mutations in 10% (to 50%) of BCCs<br>ERK/MAPK<br><br>NF-κB/ COX-2 moderate | Ristich VL *et al.*, 2006[11]<br>Freinkel RK and Woodley DT, 2001[225]<br>Jadali A *et al.*, 2010[86]<br>Kodaz H *et al.*, 2017[60]<br>Ming LM, 2007[226]<br>Ivanova PV *and* Maneva AI, 2018[27] |
| | | | Early mutation in 14-3-3σ (down-regulation)<br>activation of HH/ GLI pathway<br>activation of Wnt/β-catenin<br><br>Mutations in p53 (TP53) downregulation - Apoptosis | Lodygin D *et al.*, 2003[129]<br>Nitzki F *et al.*, 2012[227]<br>Pandolfi S *and* Stecca B, 2015[45]<br>Arun SN *et al.*, 2010[65]<br>Papanikolaou S *et al.*, 2012[228]<br>Ivanova PV *and* Maneva AI, 2018[27] |
| 6. | Spinocellular carcinoma (SCC) | Downregulated | (E-cadherin)Wnt /β-catenin<br><br>PI3K gene (PIK3CA) amplification and low-level copy number increase<br><br>NF-κB/ Cox-2<br>Increased MMPs expression<br>regulate Bcl-2 expression<br>regulate the expression of P-gp<br>regulate HIF-1α, GLUT-1 and LDHA expression<br>regulate phosphorylation of p38 MAPK<br>ERK1/2 and NF-κB, transactivating EGFR | Ristich VL *et al.*, 2006[11]<br>Suh Y *et al.*, 2014[46]<br>Rothenberg SM *and* Elisun LW, 2012[53]<br>Doma E *et al.*, 2013[181]<br>Kozaki K *et al.*, 2006[186]<br>Ivanova PV *and* Maneva AI, 2018 *(Table 2)*[27]<br>Smith A *et al.*, 2013[229]<br>Ivanova PV *and* Maneva AI, 2018, 2019[27,32]<br>Wang JN *et al.*, 2019[141]<br>Chen J *et al.*, 2018[142]<br>Zhang L *et al.*, 2018[140] |
| | | | EGFR is overexpressed, amplified, or constitutively activated by ligand interaction or mutation<br>Ras is frequently activated (Ras-Raf-ERK1/2), but with low frequency of mutations<br>Mutations in $p16^{INK4A}$, ($p21^{Waf1/Cip1/Sdi1}$, $p27^{kip1}$) hypermethylation<br><br>Mutations in p53 (TP53) - apoptosis | Yavrouian EJ *and* Sinha UK, 2012[230]<br>Doma E *et al.*, 2013[181]<br>Kodaz H *et al.*, 2017[60]<br>Rousseau A *and* Badoual C, 2011[231]<br>Suh Y *et al.*, 2014[46]<br>Suh Y *et al.*, 2014[46]<br>Ivanova PV *and* Maneva AI, 2018[27] |

Table 2

**PKD isoforms expression in various cancers.**

| Cancer: | PKD1 | PKD2 | PKD3 | References: |
|---|---|---|---|---|
| Breast | ↓ Downregulated in highly-invasive breast cancers (promoter methylation)<br><br>↑ High *PRKD1* mRNA levels are predictive of a poorer prognosis in both the entire cohort and the TNBC subgroup (triple-negative breast cancers)<br>- PKD1 is still expressed in less aggressive BC cells that are estrogen-receptor (ER) positive (cells are depleted of PKD1 they become aggressive and highly motile)<br>-PKD1 maintains the epithelial phenotype iin BC<br><br>- In breast cancer, a study from Kim and coll. showed that PKD1 can induce chemoresistance in cells | - Unchanged<br><br>Supports breast cancer development by promoting cell migration, proliferation and multi-drug resistance<br><br>In TNBC PKD2 and PKD3 have been shown to promote oncogenic progression (proliferation, invasion) and multidrug resistance (reactivation of PKD1 or inhibition of PKD2 and PKD3).<br><br>ELAVL1 was identified as a common hub-node in networks of PKD2/3-regulated phosphoproteins and genes.<br><br>PRKD inhibitor CRT0066101 exhibits anti-TNBC effects via modulating a phosphor-signaling network and inhibiting the phosphorylation of many cancer-driving factors, including p-MYC(T58/ S62), p-MAPK1/3(T202/Y204), p-AKT(S473), p-YAP(S127), and p-CDC2(T14) | ↑ increased expression with increasing aggressiveness<br><br>↑ TNBC, the most aggressive forms of breast cancer, have been reported to express PKD2 and PKD3, but not PKD1 (as oestrogen receptor-negative)<br><br>In TNBC PKD3 mRNA<br>PKD2 and PKD3 have been shown to promote oncogenic progression (proliferation, invasion) and multidrug resistance (reactivation of PKD1 or inhibition of PKD2 and PKD3).<br><br>ELAVL1 was identified as a common hub-node in networks of PKD2/3-regulated phosphoproteins and genes.<br>PRKD inhibitor CRT0066101 exhibits anti-TNBC effects via modulating a phosphor-signaling network and inhibiting the phosphorylation of many cancer-driving factors, including p-MYC(T58/ S62), p-MAPK1/3(T202/Y204), p-AKT(S473), p-YAP(S127), and p-CDC2(T14) | Durand N *et al.*, 2016[38,12]<br>Storz P, 2018[37]<br><br>Spasojevic C *et al.*, 2018 [173]<br><br>Borges S *and* Storz P, 2013[232]<br><br>Kim DY *et al.*, 2016[233]<br><br>Liu Y *et al* ., 2019[175,176] |
| Prostate | ↑ Elevated in human prostate carcinoma tissues compared to normal prostate epithelial tissue;<br><br>↓ PKD1 expression – more malignant phenotype of pancreatic cancer<br><br>**↓Androgen-independent tumors** | PKD2 and PKD3 promoted NF-κB signaling and urokinase-type plasminogen activator (uPA) expression.activation, which are crucial for prostate cancer invasion. Silencing of endogenous PKD2 and/or PKD3 markedly decreased prostate cancer cell migration and invasion. PKD2 and PKD3 | ↑ elevated in human prostate carcinoma;<br><br>↑Advanced-stage tumors were found to have increased PKD3 nuclear accumulation; strong correlation between PKD3 nuclear localization and increasing tumor grade | LaValle C *et al.*, 2010[1] ]<br>Zou *et al* , 2012[234]<br><br>Jaggi M *et al.*, 2003[144]<br>Storz P, 2018[37]<br><br>Du C *et al.*, 2009[157]<br>Du C *et al.*, 2010[131] |

| | | | | |
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| | **showed reduced PKD1 expression** | promoted the activity of uPA and MMP-9.<br><br>PKD2/3 contributed to mast cells recruitment and tumor angiogenesis in the prostate cancer microenvironment. PKD2/3 interacted with Erk1/2 and activated Erk1/2 or NF-κB signaling pathway, leading to AP-1 or NF-κB binding to the promoter of scf, ccl5 and ccl11. | PKD2/3 contributed to mast cells recruitment and tumor angiogenesis in the prostate cancer microenvironment. PKD2/3 interacted with Erk1/2 and activated Erk1/2 or NF-κB signaling pathway, leading to AP-1 or NF-κB binding to the promoter of scf, ccl5 and ccl11. | Du C *et al.*, 2012[156,234]<br><br>Xu W *et al.*, 2019[155] |
| Pancreas | PKD1 is only expressed in islets of Langerhans and pancreatic ducts<br><br>↑ Upregulate expression of PKD1 in ADM (acinar-to-ductal metaplasia), activating the Notch and NF-kB pathway<br><br>In pre-neoplastic lesions - PKD1 expression and activity can be detected<br><br>- Lowered PKD1 expression in pancreatic cancer<br>↑ Overexpression of PKD1 in pancreatic cancer | -<br><br>- Unchanged PKD2 expression in ADM<br><br>In pre-neoplastic lesions - not involved<br><br>In order to metastasize tumor cells need to undergo an isoform switch to expression of PKD2:<br>↑ Overexpression of PKD2 in Panc89 cells led to a robust increase in expression and secretion of MMP-7 and MMP-9 (enhances invasion) | Only PKD3 is expressed in acinar cells<br><br>↓ Down-regulate expression of PKD3 in ADM<br><br>In pre-neoplastic lesions - not involved | Liou G *et al.*, 2015[56]<br><br>Liou G *et al.*, 2015[56]<br>Döppler H *et al.*, 2016[22]<br>DöpplerH *and* Storz P, 2017 [57]<br><br>Eiseler T*et al.*, 2012[58]<br><br>Liou G *et al.*, 2015[55,56]<br>Storz P, 2018[37]<br>Wille C *et al.*, 2013[235] |
| Skin (HNC) | ↑ Expression in BCC<br><br>↓ Downregulated in NHSCC<br>The only PKD isoform whose protein and transcript levels were persistently downregulated in HNSCC cell lines, as a result of a combination of genetic and epigenetic alterations. Increased PKD1 expression after Doxycyclin treatment, not influencing proliferation and motility of HNSCC. Dox-treated PKD1-c1 mice also showed | There is no data<br><br>PKD2 mRNA was upregulated in seven out of ten tumors vs normal in patient-paired HNSCC tissue specimens. It is possible that PKD2 plays a predominant role in the growth, survival, and motility of HNSCC cells, and these functions have compensated the loss of PKD1 in tumors. Increased PKD2 or PKD3 expression in tumor vs. normal tissue. | There is no data<br><br>PKD3 was minimally expressed in the control and in almost all HNSCC cell lines examined (increased PKD2 or PKD3 expression in tumor vs. normal tissue). | Ristich V *et al.*, 2006[11]<br><br>Zhang L *et al.*, 2018[140] |

| | | | | |
|---|---|---|---|---|
| | elevated p-EKR1/2 and reduced IκBα, indicative of the activation of the MEK/ERK1/2 and the NF-κB signaling pathways. In contrast, the PI3K/Akt signaling pathway was not affected since p-Akt level was not altered. Accordingly, IHC staining showed increased cell proliferation (Ki67) in tumor explants of the Dox-treated PKD1-c1 group as compared with the controls Thus, overexpression of PKD1 promoted the growth of HNSCC tumor xenografts.<br>- Iinhibiting the expression of PKD1 in SCC-25 cells by RNA interference could inhibit the growth and promote the apoptosis of SCC-25 cells via downregulating Bcl-2 expression; downregulate the expression of P-gp, thereby decreasing both the IC50 and resistance index of paclitaxel. A potential therapeutic target for oSCC.<br>- PKD1 by shRNA interference - the growth of SCC25 cells under hypoxia was significantly decreased, as well as the expression of HIF-1α, while the percentage of apoptotic SCC25 cells was increased. Furthermore, stable silencing of PKD1 in SCC25 cells under a hypoxic condition decreased glucose uptake, lactate production and glycolytic enzyme (GLUT-1 and LDHA) expression, as well as | | | Wang JN *et al.*, 2019[141]<br>Chen J *et al.*, 2018[142] |

| | reduced the phosphorylation of p38 MAPK. | | | |
|---|---|---|---|---|

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## ЖЕЛЕЗЫ И ЛИМФОИДНЫЕ СТРУКТУРЫ МОЧЕВОГО ПУЗЫРЯ ЧЕЛОВЕКА В ОБЛАСТИ СФИНКТЕРОВ

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## THE GLANDS AND LYMPHOID STRUCTURES OF THE HUMAN URINARY BLADDER IN SPHINCTERS ZONES

DOI: 10.31618/ESSA.2782-1994.2021.4.68.26

**Abstarct**. We have studied the glands and lymphoid structures of urinary bladder of human in sphincters zones with morphological methods on 38 preparations of different ages (from newborn till to senile period). the glands (by RD Sinelnikov method) and lymphoid structures were investigated by macro-microscopic methods after stained by Hellman method. The micro preparations incisions sphincters zones of a urinary bladder in the thickness 5-7 microns were stained with hematoxylin-eosin, by Van-Gizon, Veygert and Kreyberg methods. In the sphincters zones the glands are in close microtopographical relations with lymphoid structures (lymphoid nodules and diffuse lymph tissue) and as a rule the glands are located densely. Without dependence from age, microanatomy parameters of the glands and lymphoid structures of the sphincters zones of urinary bladder are more than in outside. Dimensional indicators at right and left ureters sphincters almost correspond. It is connected by a similar design. At senile age, lymphoid structures in sphincters of a urinary bladder are single or absent.

**Аннотация**. Морфологическими методами изучили железы и лимфоидные структуры в области сфинктеров на 38 препаратах мочевого пузыря у людей разного возраста (от новорожденности до старческого периода). Железы были исследованы макромикроскопическим методом Р.Д. Синельникова, а лимфоидные структуры после окраски по Хельману с гематоксилином Гарриса. Железы слизистой оболочки при последней окраске не выявляются. Для гистологического изучения этих структур, срезы толщиной 5-7 мкм окашивали гематоксилином-эозином, по Ван Гизону, по Вейгерту, по Крейбергу, выборочно по ШИК-реакцию и серебрением по Гримелиусу. Размерные показатели и плотности расположения желез и лимфоидных структур сфинктерных зон мочевого пузыря больше, чем в внесфинктерных. Полученные данные лимфоидных структур внутреннего сфинктера мочевого пузыря, соответствуют таковым размерным показателям мочеточниковых сфинктеров. В области правого и левого мочеточниковых сфинктеров размерные показатели лимфоидных структур почти соответствуют друг другу, что, вероятно, обусловлено принципиально аналогичной конструкцией этих зон. В старческом возрасте лимфоидные узелки в сфинктерах мочевого пузыря единичны или отсутствуют.

*Key words: morphology, glands, lymphoid nodules, urinary bladder, sphincters*

*Ключевые слова: морфология, железы, лимфоидные структуры, мочевой пузырь, сфинктеры, постнатальный онтогенез*

### ВВЕДЕНИЕ

Известно, что сфинктерные зоны состоять из совокупности специально организованных структур позволяющей регулировать сообщения между частями полых внутренних органов, регулируют перемещение содержимого, выполняя антирефлюксную функцию [4,5,8,9]. Сфинктерный аппарат полого органа представляет в виде складок слизистой оболочки вместе с содержимыми, т.е. сосудисто-нервными пучками, малыми железами и лимфоидными узлами [9,10]. Малые железы и лимфоидные структуры в зонах сфинктеров полых

внутренних органов в том числе мочевого пузыря, играют значительную роль в жизнедеятельности их в норме и патологии [1-3,10-11,15]. Эти особенности связаны с высокой структурно-функциональной лабильностью малых желез и лимфоидных структур в стенке полых органов [10,14].

О морфологических особенностях лимфоидных структур мочевого пузыря и ее сфинктерных зон, являющихся структурным компонентом стенки органа, известно очень немного, что, очевидно, связано с незаслуженным отсутствием интереса к этому вопросу, не исследованы размерно-количественные показатели этих структур в разных возрастных периодах постнатального онтогенеза.

**ЦЕЛЬ ИССЛЕДОВАНИЯ**

Цель исследования явилось получение морфологических данных о железы и лимфоидные структуры сфинктеров мочевого пузыря, т.е. внутреннего сфинктера уретры, а также правого и левого мочеточниково-мочепузырных сфинктеров у людей разного возраста в постнатальном онтогенезе.

**МЕТОДЫ ИССЛЕДОВАНИЯ**

В соответствии с целю работы разными морфологическими методами изучены железы и лимфатические структуры обоих мочеточниково-мочепузырных сфинктеров и внутреннего сфинктера уретры человека. Исследование проводилось на 38 тотальных препаратах мочевого пузыря на трупе людей от новорожденности до старческого периода постнатального онтогенеза. Железы были исследованы макромикроскопическим методом Р.Д. Синельникова, а лимфоидные структуры после окраски по Хельману с гематоксилином Гарриса. Железы слизистой оболочки при последней окраске не выявляются. Для гистологического изучения этих структур, срезы толщиной 5-7 мкм окашивали гематоксилином-эозином, по Ван Гизону, по Вейгерту, по Крейбергу, выборочно по ШИК-реакцию и серебрением по Гримелиусу. Для биометрии данных исследования использовали ЭВМ IBM 486 SX33 с помощью пакета прикладных программ «Морфолог», работая в среде Windows.

**РЕЗУЛЬТАТЫ И ИХ ОБСУЖДЕНИЕ**

Железы мочевого пузыря имеют четкие контуры. Они могут располагаться концентрировано, когда между железами остаются небольшие промежутки, меньшие по размерам, чем начальный или альвеолярный отдел железы. В области мочеточниковых сфинктеров мочевого пузыря и внутреннего сфинктера уретры вне зависимости от возраста и пола, образуются «железистые муфты» - скопления начальных отделов желез (рис.1). В этих зонах начальные отделы сконцентрированы, расположены настолько плотно, что даже при макромикроскопическом препарировании (препаровальной иглой) установить принадлежность начального отдела той или иной железе не представляется возможным. Возле сфинктерных зон железы малочислены, отсутствуют, или же более мелкие при визуальном изучении, по сравнению с областью сфинктера.

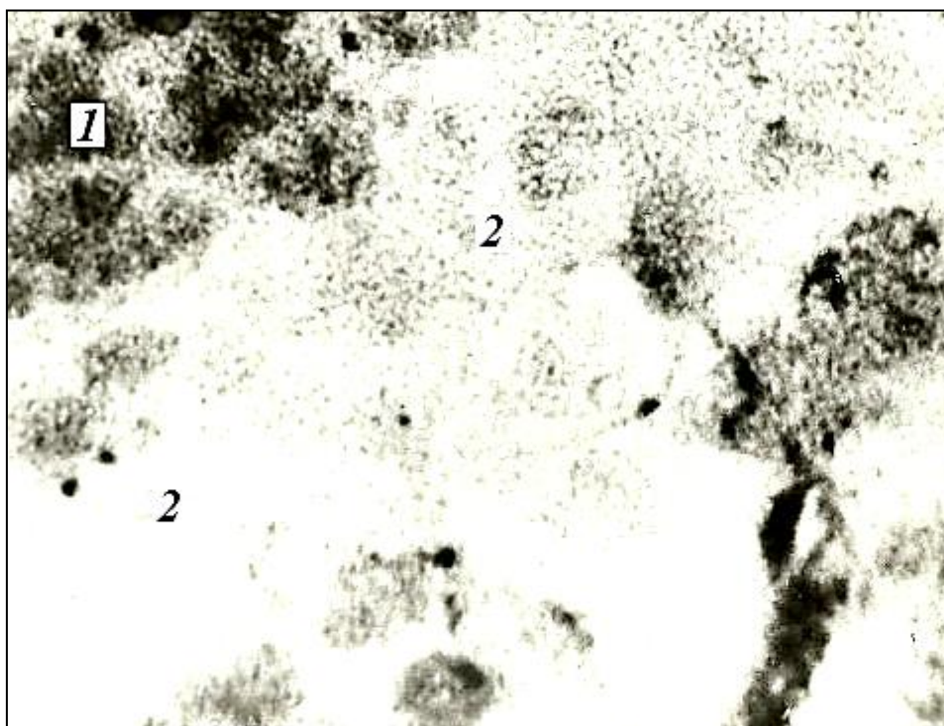


*Рис. 1. Скопление начальных отделов желез в области внутреннего сфинктера уретры у мужчины 34 лет. 1 - скопление начальных отделов; 2 - внесфинктерная зона. Окраска по Р.Д. Синельникову. Ув.25х.*

Морфологические особенности желез и лимфатических структур сфинктерных зон мочевого пузыря зависит от возраста. Так как, в разных этапах постнатального онтогенеза размерные показатели желез и лимфатических структур в сфинктерные зоны мочевого пузыря существенно меняется. Максимальных значений показатели желез достигают в 1-м периоде зрелого возраста, а лимфатических структур в раннем детском возрасте.

Согласно полученным данным (таблица 1), в зоне внутреннего сфинктера уретры толщина альвеолярного отдела и количество альвеолярных частей (альвеол) по сравнению с внесфинктерной зоны, у новорожденных детей, в раннем детстве и у подростков больше в 1,1-1,2 раза ($p < 0,05$), в 1-м периоде зрелого возраста - в 1,2-1,3 раза ($p < 0,05$), а в старческом возрасте - в 1,3-1,6 раза ($p < 0,05$).

Таблица 1.

**Некоторые размерные показатели строения желез в зоне мочеточниково-мочепузырных сфинктеров (по данным микроскопии; X + Sx; min-max).**

| Возраст и зона расположения желез | n | Наименование показателей, размерность | | |
|---|---|---|---|---|
| | | Длина (mm) | Ширина (mm) | Количество альвеол на срезе |
| **Новорожденные** | 9 | | | |
| внутренний сфинктер уретры | | 0,13±0,01 | 0,12±0,01 | 10,5±0,4<br>8-12 |
| правый мочеточниковый сфинктер | | 0,13± 0,01 | 0,13±0,01 | 10,5±0,4<br>9-12 |
| левый мочеточниковый сфинктер | | 0,12± 0,01 | 0,12±0,01 | 10,2±0,4<br>9-12 |
| около сфинктерная зона | | 0,11±0,01 | 0,10± 0,01 | 8,2±0,5<br>7-12 |
| **Ранний детский возраст** | 8 | | | |
| внутренний сфинктер уретры | | 0,19+0,01 | 0,17+0,01 | 14,5+0,8<br>11-18 |
| правый мочеточниковый сфинктер | | 0,17±0,01 | 0,15±0,01 | 14,5±0,9<br>11-18 |
| левый мочеточниковый сфинктер | | 0,17±0,01 | 0,15±0,01 | 12,6±0,9<br>11-18 |
| около сфинктерная зона | | 0,13±0,01 | 0,12±0,01 | 12,6±1,1<br>8-18 |
| **Подростковый** | 7 | | | |
| внутренний сфинктер уретры | | 0,27±0,01 | 0,22±0,01 | 26,5±0,9<br>22-30 |
| правый мочеточниковый сфинктер | | 0,25±0,01 | 0,22±0,01 | 26,5±1,1<br>22-30 |
| левый мочеточниковый сфинктер | | 0,24±0,01 | 0,22±0,01 | 25,9±0,9<br>23-30 |
| около сфинктерная зона | | 0,19±0,01 | 0,17±0,01 | 17,9±0,8<br>13-30 |
| **1-й зрелый период** | 7 | | | |
| внутренний сфинктер уретры | | 0,34+0,01 | 0,26+0,01 | 29,5+0,7<br>26-32 |
| правый мочеточниковый сфинктер | | 0,28+0,01 | 0,27+0,01 | 26,5+0,8<br>24-32 |
| левый мочеточниковый сфинктер | | 0,29+0,01 | 0,27+0,01 | 25,0+0,9<br>23-32 |
| около сфинктерная зона | | 0,22± 0,01 | 0,21± 0,01 | 24,0±1,7<br>13-32 |
| **Старческий** | 7 | | | |
| внутренний сфинктер уретры | | 0,16±0,01 | 0,14±0,01 | 18,5±0,5<br>15-20 |
| правый мочеточниковый сфинктер | | 0,16±0,01 | 0,14±0,01 | 18,5±0,5<br>15-20 |
| левый мочеточниковый сфинктер | | 0,18+0,01 | 0,15+0,01 | 18,0+0,6<br>15-21 |
| около сфинктерная зона | | 0,10+0,01 | 0,09+0,01 | 14,0+1,2<br>7-20 |

**Примечание:**
1. n – число наблюдений;
2. mm –показатель длины;
3. $X \pm S_x$ – среднее арифметическое вычисление;
4. Около сфинктерная зона – участок слизистой оболочки мочевого пузыря на 1 см расстоянии сфинктеров.

Согласно нашим данным, лимфоидные образования в стенках мочевого пузыря в норме представлены лимфоидными узелками (всегда без центров размножения) и диффузной лимфоидной тканью, располагающимися преимущественно в собственной пластинке слизистой оболочки (рис. 2). На тотальных препаратах, лимфоидные узелки окрашиваются в виде темных (черных, темно-синих) анатомических образований, имеющих преимущественно четкие контуры, при этом стенка органа несколько более светлая. Форма лимфоидных узелков разнообразная: овальная округлая, неправильная. Они располагаются поодиночке, образуют скопления или незначительные прямые, с изгибами, прерывистые цепочки. Возле узелков располагаются участки слизистой оболочки, где лимфоидные узелки отсутствуют («безлимфоидные участки»). Вместе с тем, вокруг устьев мочеточников лимфоидные узелки образуют неправильной формы кольцо, а в области шейки мочевого пузыря (внутренний сфинктер уретры) постоянно формируют скопления - «лимфоидные муфты», возле которых находятся «безлимфоидные участки» слизистой оболочки и визуально более мелкие, по сравнению с сфинктерной зоной, лимфоидные узелки (рис.2). Лимфоидные узелки и диффузная лимфоидная ткань постоянно находятся возле отверстий мочеточников и рядом с отверстием мочеиспускательного канала возле выводных протоков на всем их протяжении и рядом с устьем протока. Некоторые узелки формируют короткие и прерывистые цепочки. Размеры лимфоидных узелков неравномерны, как и расстояние между соседними узелками. Вероятно, в сфинктерных зонах слизистая оболочка благодаря повышенной активности мускулатуры стенки [5-6,8-11,13], интенсивно соприкасается с содержимым мочевого пузыря и субстанциями в моче. Это в свою очередь обусловливает необходимость дополнительной защиты внутреннего слоя органа и слизистого секрета желез [4].

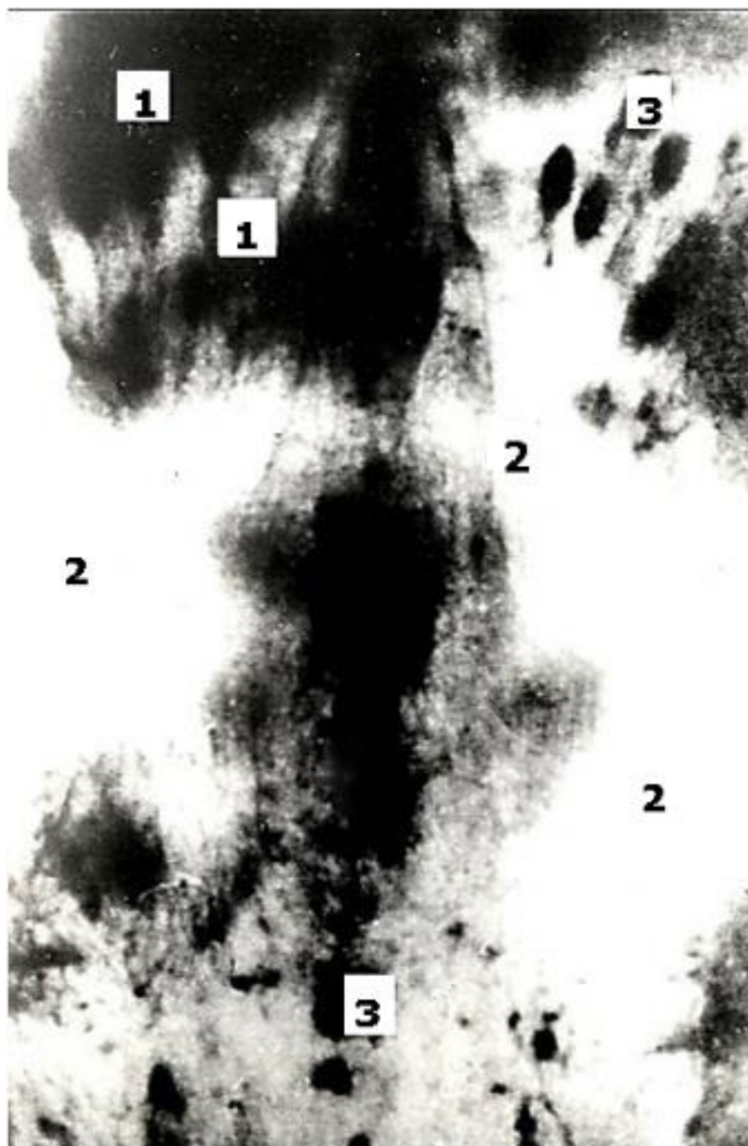


*Рис.2. Лимфоидные узелки в области внутреннего сфинктера уретры мужчины 22 лет.*
*1-скопление лимфоидных узелков в области сфинктера; 2-внесфинктерные зоны;*
*3-мелкие лимфоидные узелки возле сфинктера. Окраска по Хельману. Ув.10х.*

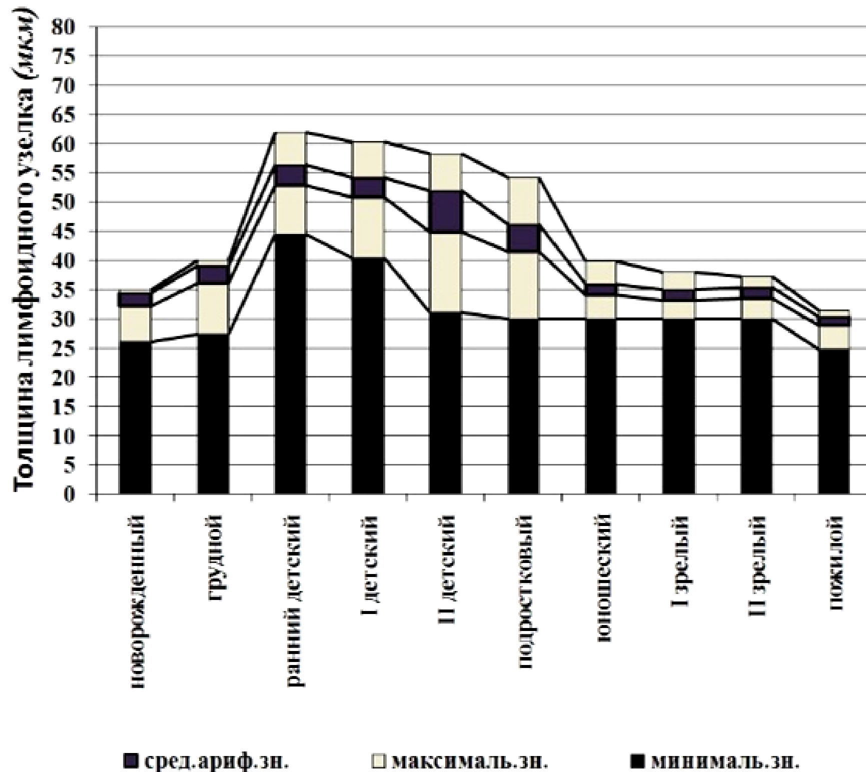


*Диаграмма 1. Толщина лимфоидного узелка во внутреннем сфинктере уретры в постнатальном онтогенезе.*

В области треугольника, т.е. в задней стенке нижней трети мочевого пузыря, где расположены все сфинктеры, количество лимфоидных узелков больше, чем в верхней и средней части органа. В области сфинктеров мочевого пузыря количество лимфатических узелков в раннем детском возрасте достигает онтогенетического максимума. Этот показатель увеличивается в раннем детском возрасте в 1,7 раза (p <0,05), по сравнению с этим возрастом. По сравнению с ранним детским возрастом показатель уменьшается у подростков и в 1-м периоде зрелого возраста в 1,3 (p <0,05), в пожилом возрасте в 1,4 (p <0,05) и в старческом возрасте в 3,1 раза (p <0,05). Вне зависимости от возраста в зоне всех сфинктеров количество и размерные показатели лимфоидных узлов больше, чем вне сфинктеров. Так как, во внутреннем сфинктере уретры количество лимфоидных узлов в 1,1-1,5 раза (p<0,05) больше, чем в внесфинктерной зоне. Следует добавить, что этот показатель лимфатического узла в области правого и левого мочеточниковых сфинктеров почти соответствуют друг другу. Это соответствие, вероятно, обусловлено аналогичной конструкцией этих сфинктеров, трехслойным строением мускулатуры, схожего состава сосудов и нервов. Толщина лимфатических узелков мочевого пузыря около внутреннего сфинктера уретры увеличивается в раннем детском возрасте в 1,5 раза (p<0,05), достигая онтогенетического максимума (диаграмма 1). По сравнению с ранним детским возрастом, данный показатель уменьшается у подростков и в 1-м периоде зрелого возраста в 1,2 раза (p> 0,05), в пожилом возрасте – в 2,0 раза (p <0,05). В старческом возрасте лимфоидные узелки в этом отделе стенки мочевого пузыря единичны (или отсутствуют).

Общий принцип макромикроскопической и гистологической структурной организации лимфоидных узлов сфинктерных зон мочевого пузыря соответствует со свойством малых желез стенок полых внутренних органов [2,5-7,13-14].

**ЗАКЛЮЧЕНИЕ**

1. На протяжении постнатального онтогенеза морфометрические показатели желез и лимфоидных структур сфинктерных зон мочевого пузыря существенно различаются, т.е. максимальных значений эти показатели у желез достигают в 1-м периоде зрелого возраста, а у лимфатических структур в раннем детском возрасте.

2. В зоне сфинктеров мочевого пузыря человека вне зависимости от возраста, размерные показатели желез и лимфатических узелков больше, чем во внесфинктерной зоне.

3. Полученные размерные данные желез и лимфоидных узлов внутреннего сфинктера мочевого пузыря, соответствуют таковым показателям мочеточниковых сфинктеров. В области правого и левого мочеточниковых сфинктеров размерные показатели почти соответствуют друг другу, что, вероятно, обусловлено принципиально аналогичной конструкцией.

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#4(68), 2021 part 4

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198320, Санкт-Петербург,

Город Красное Село,

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д. 44, к. 1, литера А

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