EDITORIAL

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Protein kinase D1: gatekeeper of the epithelial phenotype and key regulator of cancer metastasis?

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The development of epithelial cancer and subsequent metastasis goes along with loss of cellular polarity and cell–cell connections (Gandalovicova *et al*, 2016). Epithelial-to-mesenchymal transition (EMT) and reorganisation of the extracellular matrix by secreted matrix metalloproteinases (MMPs) are key mechanisms in the separation of tumour cells from the surrounding cells, and their dissemination to distant locations in the body (Nistico *et al*, 2012). Recent work by several groups suggests that protein kinase D (PKD) enzymes serve crucial functions in regulating multiple processes in maintaining the epithelial phenotype. A new article in this issue of the *British Journal of Cancer* (Ganju *et al*, 2018) has shed further light on this role.

The PKD family of serine/threonine kinases consists of three members, PKD1, PKD2, and PKD3, which phosphorylate a broad spectrum of targets and control multiple functions within cells (Fu and Rubin, 2011). Although, with respect to tumour formation, all three isoforms have been implicated in regulating cancer cell survival and proliferation, the initiation of the metastatic process requires a shift in PKD isoform expression (Durand *et al*, 2015). For example, in tumours of the breast, a transition from a less aggressive to a more aggressive metastatic phenotype is characterised by PKD1 gene promoter methylation and downregulation, whereas PKD3 is upregulated in its expression (Borges *et al*, 2013, 2015). This is because PKD1 blocks cell motility, whereas PKD3 seems to drive this event (Durand *et al*, 2015).

In tumour cells, PKD1 negatively regulates the motile phenotype of cells through multiple mechanisms involving the regulation of focal adhesion dynamics (Jaggi *et al*, 2005; Durand *et al*, 2016), actin reorganisation dynamics at the leading edge (Eiseler *et al*, 2009b; Spratley *et al*, 2011; Doppler *et al*, 2014), and filopodia formation (Doppler *et al*, 2013) (Figure 1). This is achieved through phosphorylation of a multitude of substrates, with a net effect of complete downregulation of processes that drive cell migration and invasion. For example, 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 (Bravo-Cordero *et al*, 2013; Mizuno, 2013). 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) (Eiseler *et al*, 2009b), as well as phosphorylation-mediated activation of p21-activated kinase 4 (PAK4), which is an upstream kinase of the cofilin kinases LIMK1/2 (Spratley *et al*, 2011; Doppler *et al*, 2014). Focal adhesion dynamics and filopodium formation are regulated by PKD1 through phosphorylation of phosphatidylinositol-4-phosphate 5-kinase type-1γ (PIP5K1γ) and vasodilator-stimulated phosphoprotein (Doppler *et al*, 2013; Durand *et al*, 2016). In addition to this, PKD1 blocks extracellular matrix degradation by decreasing the expression of MMPs (Eiseler *et al*, 2009a), whereas the two other PKD isoforms induce their expression (LaValle *et al*, 2012; Wille *et al*, 2014).

PKD1 also maintains the epithelial phenotype of cells by negatively regulating key molecules that control EMTs. For example, PKD1 phosphorylates Snail (SNAI1), a transcriptional repressor for *CDH1* (encoding E-cadherin) in the nucleus of breast cancer cells (Bastea *et al*, 2012), leading to nuclear export (Du *et al*, 2010) and subsequent proteasomal degradation of Snail (Zheng *et al*, 2014). In addition to regulating E-cadherin at the gene level, Jaggi and colleagues demonstrated in their previous work that PKD1 regulates the nuclear/cytosolic shuttling of β-catenin (Du *et al*, 2009), and therefore controls the formation of E-cadherin-mediated cell–cell contacts (Jaggi *et al*, 2005).

In this issue of the *British Journal of Cancer*, Ganju *et al* (2018) now expand the insight into this functional aspect of PKD1-mediated regulation of the epithelial phenotype. They discovered

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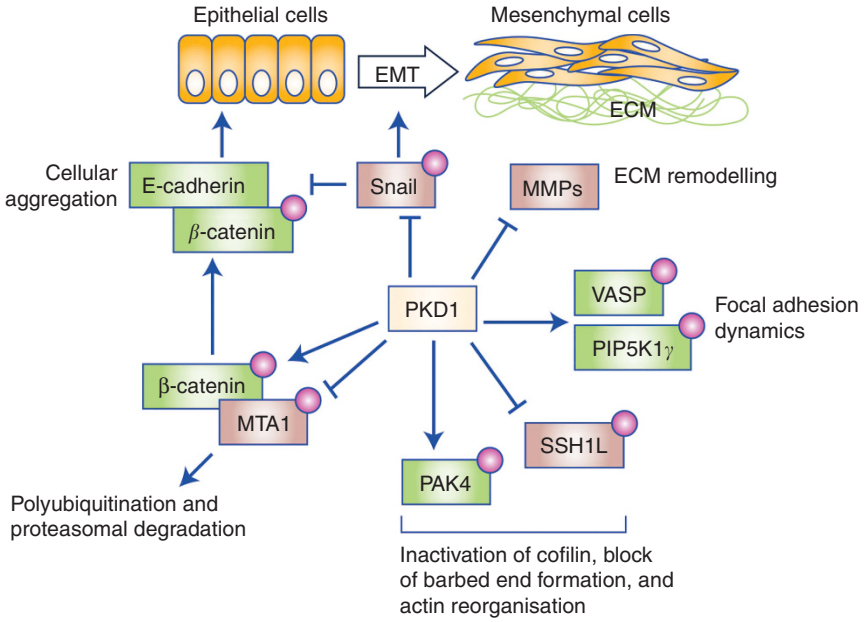

Figure 1. Roles of PKD1 in maintaining the epithelial phenotype. Shown are some of the key events that are regulated by PKD1 and that prevent EMT and decrease cell motility. PKD1 blocks actin cytoskeleton rearrangements needed for cell movement through phosphorylation of SSH1L (inactivation) and PAK4 (activation), with a net effect of inhibiting cofilin. PKD1 also affects filopodia formation and length through phosphorylation of VASP, as well as focal adhesion dynamics by targeting PIP5K1γ and VASP. EMT and remodelling of the extracellular matrix are regulated through phosphorylation and proteasomal degradation of Snail, and by inhibition of MMP expression. In addition to increasing E-cadherin expression through inactivation of Snail, PKD1 also regulates the formation of E-cadherin-mediated cell–cell connections through phosphorylation of MTA1 and β-catenin, leading to MTA1 proteasomal degradation and β-catenin location to the E-cadherin complexes. EMT=epithelial-to-mesenchymal transition; MMPs=matrix metalloproteinases; MTA1=metastasis-associated protein 1; PKD1=protein kinase D1.

metastasis-associated protein 1 (MTA1) as a novel substrate for PKD1, and show that PKD1-mediated phosphorylation of MTA1 triggers polyubiquitination and proteasomal degradation. Moreover, they propose that this leads to the shuttling of PKD1/β-catenin complexes to E-cadherin, with the effect of stabilising cell–cell contacts. In addition to providing this mechanistic insight at a cellular level, Ganju *et al* also demonstrated a reverse correlation between PKD1 and MTA1 expression in samples of human prostate, colon, and breast cancers, in which PKD1 expression decreased and MTA1 expression increased, with progressed tumour grade or stage. Since PKD1 has such marked negative-regulatory effects on cell–cell aggregation, EMT, cell migration, and invasion, it is not surprising that it is epigenetically downregulated in many invasive cancers.

The present work by Ganju *et al* provides additional strong *in vitro* and *in vivo* evidence that PKD1 indeed is a gatekeeper of the epithelial phenotype and prevents cell migration and invasion at multiple levels; however, important questions remain. For example, with respect to the dual roles of PKD isoforms in cancer progression, it remains unclear how PKD1 can have such profound negative-regulatory effects on cell motility, whereas PKD3 seems to act in the exact opposite manner. It may be explained by differences in the regulatory elements of these kinases such as the PDZ-binding motif at the C terminus, which only occurs in PKD1 and PKD2, but not in PKD3 (Durand *et al*, 2015). Moreover, the different functions between PKD isoforms in the invasive stages of cancer also leads to the question of whether pan-PKD inhibitors are suitable tools for therapy. This, in part, has been answered for pancreatic, bladder, colorectal, and breast cancers (Harikumar *et al*, 2010; Wei *et al*, 2014; Borges *et al*, 2015; Li *et al*, 2017). In triple-negative and oestrogen receptor-negative invasive breast cancers, for example, which express PKD2 and PKD3, a pan inhibitor (CRT0066101) in mice has been shown to reduce primary tumour growth as well as metastasis to the lung (Borges *et al*, 2015); however, effects of this inhibitor on breast cancers that also express PKD1 have not yet been tested. Although, one would expect that treatment of primary tumours will be affected in their proliferation, which is regulated by all three PKDs, one could assume that inhibition of PKD1 would conversely lead to higher motility of cancer cells. However, the simultaneous inhibition of PKD2 and PKD3, which are both needed for motility and invasiveness, should prevent spreading and metastasis.

In summary, while the exact roles of PKD isoforms in many aspects of tumour initiation, progression, and metastasis are still ill-defined, the work by Ganju *et al* now adds important *in vitro* and *in vivo* findings, which contribute to our understanding of how PKD1 maintains the epithelial phenotype. This information is also needed in order to judge the use of PKD inhibition strategies for several human cancers, and to evaluate the patient groups that could benefit most from therapy with pan-PKD inhibitors.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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