Arresting times for PTEN

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The tumour suppressor PTEN (phosphatase and tensin deleted on chromosome 10) is an important regulator of cell proliferation and migration. In this issue of *The EMBO Journal*, Lima-Fernandes and colleagues show that the universal signalling scaffold protein, β -arrestin dynamically interacts with PTEN, through a phosphorylation-controlled switch. This protein–protein interaction profoundly influences the ability of PTEN to regulate both cell proliferation, through activation of its lipid phosphatase activity, and enhance cell migration by unblocking the inhibitory effect of the PTEN C2 domain and by recruiting activated ROCK into a β -arrestin/PTEN ‘signalosome’.

Understanding the myriad of mechanisms that contribute to cell proliferation and migration, and how these are modified in various cancers, will point the way to new, personalized diagnostics and therapeutics. The tumour suppressor PTEN (phosphatase and tensin deleted on chromosome 10) regulates numerous key cellular functions including proliferation and migration with its relevance to normal cell physiology exemplified by the fact that its gene is frequently deleted or mutated in a wide variety of human cancers (Leslie and Downes, 2002; Salmena *et al*, 2008). In this edition of *The EMBO Journal*, Lima-Fernandes *et al* (2011) show, for the first time, that at the cross-roads controlling the proliferative and migratory determining aspects of PTEN is the universal signalling scaffold protein, β -arrestin (Defea, 2008). The discovery that β -arrestins serve as upstream signalling regulators of PTEN is likely to have important consequences for our understanding of progression and metastasis associated with certain types of cancer and to point towards the development of novel diagnostic and therapeutic approaches.

PTEN exhibits dual-specificity phosphatase activity able to dephosphorylate not only lipids, of which its major physiological substrate is phosphatidylinositol 3,4,5 trisphosphate (PIP3) but various tyrosine phosphate containing peptides. It is, however, by degrading PIP3 into phosphatidylinositol 4,5 bisphosphate (PIP2) that PTEN inhibits key proliferative and survival signals mediated by signalling through the PI 3-kinase/Akt(PKB) pathway (Leslie and Downes, 2002; Salmena *et al*, 2008).

PTEN is formed from a 186 amino-acid N-terminal domain that contributes the lipid and protein phosphatase activities together with a 217 amino-acid C-terminal domain (Figure 1A). This encompasses a 166 amino-acid C2 domain able to bind

PIP2 in a Ca^{2+} -independent manner; a PDZ protein–protein interaction domain; a PEST motif whose modification can target PTEN for degradation and, additionally, a region undergoing multisite phosphorylation that provides a potential regulatory role. The importance of the C-terminal portion of PTEN is exemplified by the fact that nearly half of the PTEN mutations found in various cancers locate to this region.

This multidomain structure allows PTEN to regulate major cellular functions not only via its lipid phosphatase activity

Figure 1 Schematic of PTEN and its function. (A) Key features of the domain structure of PTEN. (B) Agonist occupancy of a GPCR coupled to $\text{G}\alpha\text{-q/12/13}$ recruits β -arrestin with sequestered PTEN and ROCK1. This complex exhibits activated PIP3 phosphatase activity, resulting in lowered pAkt levels and diminished functioning of the PI 3-kinase/Akt cell survival/proliferative response. Additionally, the sequestration of β -arrestin in this complex negates the inhibitory effect of the PTEN C2 domain in cell proliferation and, additionally, recruits ROCK to promulgate cell migration.

but also through means that are independent of such catalytic activity. One such action is cell migration, which is clearly dependent on the C2 domain of PTEN and independent of the lipid phosphatase activity of PTEN (Raftopoulou *et al*, 2004) (Figure 1B).

The human genome encodes a very large family of G-protein-coupled receptors (GPCRs) that locate at the plasma membrane and regulate a myriad of cellular processes in a cell-type specific manner (Defea, 2008; Rajagopal *et al*, 2010). While the mode of action and the identity of the activating ligand of many of these GPCRs (so-called 'orphan' species) remains to be understood, those GPCRs that have been well-investigated appear able to undergo rapid desensitization subsequent to agonist occupancy (Defea, 2008; Rajagopal *et al*, 2010). This is conferred through the phosphorylation of the agonist-occupied GPCR by G-protein receptor kinases (GRKs). This modification triggers cytosolic β -arrestins to be recruited to the GRK-phosphorylated GPCR so as to interdict GPCR coupling to its effector guanine nucleotide regulatory protein (G-protein). However, there is an increasing body of evidence that suggests that β -arrestins can of themselves perform a variety of signalling roles through their ability to undergo conformational changes and to sequester specific proteins (Baillie and Houslay, 2005; Defea, 2008). Indeed, from the myriad of proteins that β -arrestins can potentially bind, it seems that functionally distinct sub-populations of β -arrestins exist in cells. These are defined by the distinct protein cohort they sequester as determined by spatial constraints, partnerships between sequestered proteins as well as by ubiquitination and SUMOylation modification of β -arrestins (Li *et al*, 2009). Indeed, β -arrestins can act as platforms to orchestrate proteins involved in cytoskeletal reorganization and other processes involving small GTPases.

The initial discovery of Lima-Fernandes *et al* (2011) resulted from a two-hybrid screen for β -arrestin that identified PTEN as a potential partner. The use of purified proteins and peptide array technology showed this interaction to be direct and to involve primarily the C2 domain of PTEN. Co-immunoprecipitation subsequently showed that PTEN and β -arrestins could form a complex in mammalian cells. However, particularly intriguing was the observation that β -arrestins could sequester greater amounts of PTEN truncates that lacked the N-terminal portion compared with full-length PTEN. This suggested to the authors that additional interaction sites for β -arrestins may be masked by intramolecular interactions and the possibility that access to them may be subject to dynamic regulation. In this regard, a group of phosphorylation sites within the carboxy-regulatory tail region of PTEN is believed to be able to regulate the conformation, functioning and stability of PTEN. While the nature of the protein kinases phosphorylating PTEN remains to be definitively ascertained, casein kinase 2 (CK2) has been

implicated. Notwithstanding this, Lima-Fernandes *et al* (2011) identified the dephosphorylation of Thr383 as providing the critical switch in this region that conferred increased β -arrestin binding to PTEN.

Fascinatingly, this dephosphorylation event has also been identified as allowing PTEN to exert a C2 domain-mediated inhibitory effect on cell migration (Raftopoulou *et al*, 2004). This led Lima-Fernandes and colleagues to identify an increased association between PTEN and β -arrestin following the wounding of cell monolayers and during migration. This was driven by an increase in cellular activated, GTP-bound RhoA, which promoted the association of activated ROCK into the β -arrestin/PTEN macromolecular complex. Thus, when β -arrestins interact with the C2 domain of PTEN, they rescue migration by blocking the inhibitory effect that this domain exerts on migration and by recruiting activated ROCK, which is known to be critical for cytoskeletal reorganization and migration. Intriguingly, it has also recently been shown (Anthony *et al*, 2011) that GPCR-activated β -arrestins can bind and inhibit the RhoA GAP, ARHGAP21, thereby activating RhoA activity. These data pose the possibility that β -arrestins could act as both upstream RhoA regulators and downstream RhoA effectors.

However, the effect of scaffolded β -arrestin was not confined to the C-terminal region of PTEN that it binds, as it was found to markedly activate the lipid phosphatase activity of the PTEN N-terminal catalytic unit. Intriguingly, RhoA, whose effector protein ROCK is recruited into the PTEN/ β -arrestin complex, is one of the few other proteins known to increase PTEN lipid phosphatase activity (Li *et al*, 2005). The lipid phosphatase activity of PTEN, in converting PIP₃ to PIP₂, acts as a functional antagonist to PI 3-kinase and, in so doing, prevents activation of the kinase, Akt (PKB). This negatively regulates proliferative signals dependent on the activity of Akt. Such anti-proliferative actions of β -arrestin, mediated through PTEN, were demonstrated in various cell models through either GPCR-mediated 'activation' of β -arrestins or their targeted overexpression.

These findings identify a novel link between the activation of $G_{\alpha 12/13}$ -coupled cell surface GPCR activation and PTEN function where β -arrestins serve as dynamic signalling scaffolds to regulate distinct functional outputs of PTEN that influence cell proliferation and migration (Figure 1B). An understanding of the dynamic nature of complexes involving β -arrestins and the precise cohort of proteins that form specific, β -arrestin-underpinned signalosomes, such as the one identified here with PTEN, will provide fundamental insight into cellular function as well as proliferative and metastatic processes in certain cancer types.

Conflict of interest

The author declares that he has no conflict of interest.

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