Seminars in Cell & Developmental Biology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Inherited *PTEN* mutations and the prediction of phenotype

Nicholas R. Leslie[a,*], Michel Longy[b]

[a] *Institute of Biological Chemistry, Biophysics and Bioengineering, Nasmyth Building, Heriot Watt University, Edinburgh EH14 4AS, UK*
[b] *Cancer Genetics Unit & INSERM U916, Bergonie Institute, Bordeaux University, Bordeaux, France*

ARTICLE INFO

Article history:
Received 15 October 2015
Received in revised form
21 December 2015
Accepted 21 January 2016
Available online xxx

Keywords:
PTEN
Phosphatase
Tumour suppressor
Mutation
Cancer
Autism spectrum disorder

ABSTRACT

PTEN has been heavily studied due to its role as a tumour suppressor and as a core inhibitory component of the phosphoinositide 3-kinase (PI3K) signalling network. It is a broadly expressed phosphatase which displays complexity and diversity in both its functions and regulation and accordingly, in the laboratory numerous classes of functionally distinct mutations have been generated. Inherited loss of function mutations in the *PTEN* gene were originally identified in sufferers of Cowden disease, but later shown to associate with more diverse human pathologies, mostly relating to cell and tissue overgrowth, leading to the use of the broader term, PTEN Hamartoma Tumour Syndrome. Recent phenotypic analysis of clinical cohorts of *PTEN* mutation carriers, combined with laboratory studies of the consequences of these mutations implies that stable catalytically inactive PTEN mutants may lead to the most severe phenotypes, and conversely, that mutants retaining partial function associate more frequently with a milder phenotype, with autism spectrum disorder often being diagnosed. Future work will be needed to confirm and to refine these genotype–phenotype relationships and convert this developing knowledge into improved patient management and potentially treatment with emerging drugs which target the PI3K pathway.

Contents

1. Introduction 00
2. The PTEN protein and the functional consequences of mutation 00
   2.1. The PTEN protein and its post-translational regulation 00
   2.2. Experimental analysis of the functional consequences of PTEN mutations 00
3. *PTEN* Hamartoma Tumour Syndrome and the phenotype of *PTEN* mutation carriers 00
   3.1. Human germline *PTEN* mutation 00
   3.2. Somatic mosaicism 00
4. Phenotypic variability in human *PTEN* mutation carriers and genotype–phenotype relationships 00
   4.1. Clinical studies 00
   4.2. Modelling human disease in *Pten* mutant mice 00
5. Therapeutic implications 00
6. Concluding remarks 00
   Acknowledgments 00
   References 00

1. Introduction

In 1997, PTEN, was first identified as a tumor suppressor gene and phosphatase mutated in multiple cancer types [1–3]. Soon after and based on gene localization, [4] germline PTEN mutations were shown to be causative of Cowden disease, a phenotypically complex cancer prone syndrome (OMIM: 158350) [5] and its pediatric presentation, the Bannayan Riley Ruvalcaba Syndrome (OMIM: 153480) [6]. The diversity of phenotypes now observed in PTEN mutation carriers and the numerous organs and cell types affected provide important evidence for the multiple actions of the PI3K/PTEN signalling network in the regulation of many cellular processes and sit well alongside many studies conducted in cultured cells and animal models. Here we discuss how studies of

* Corresponding author.
E-mail address: n.r.leslie@hw.ac.uk (N.R. Leslie).

http://dx.doi.org/10.1016/j.semcdb.2016.01.030

Please cite this article in press as: N.R. Leslie, M. Longy, Inherited *PTEN* mutations and the prediction of phenotype, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.01.030

patient phenotypes have linked to our understanding of PTEN function at the molecular and cellular level and conversely whether this may allow clinically useful prediction of the phenotype of PTEN mutation carriers.

The PI3K (class I phosphoinositide 3-kinase) signalling system is activated by diverse extracellular stimuli, including many growth factors, hormones including insulin, cytokines, chemokines and extracellular matrix components, which drive PI3K-dependent synthesis of phosphatidylinositol 3,4,5-trisphosphate (PIP_3) in the plasma membrane. In turn, increased PI3K activity and PIP_3 levels promote the growth, proliferation and survival of many cell types, as well as influencing cell metabolism, polarity and movement, all through effects on a large and diverse set of PIP_3-binding proteins which include the AKT protein kinases [7]. PTEN is a lipid phosphatase which directly opposes the function of the PI3Ks by dephosphorylating PIP_3. Accordingly, loss of PTEN function has been shown experimentally to cause many disturbances in cell and organism physiology, commonly linked to increases in cell growth and proliferation [8–10] and clinically PTEN loss has been identified as a driver event in the development of many sporadic cancers [11,12]. Functions for PTEN independent from its action on PIP_3 have been proposed [13–16], including protein substrates for its phosphatase activity [17–19]. The discovery of a PTEN mutation in two Cowden disease families, PTEN G129E, and characterisation showing that this mutant enzyme lacks lipid phosphatase activity, yet retains protein phosphatase activity was a key factor connecting PTEN lipid phosphatase activity with tumour suppressor function [20,21]. However, the significance of these alternative functions including protein phosphatase activity is currently unclear and the development of tumours both in these PTEN G129E carrying patients and in PTEN G129E knockin mice indicates that PIP_3-independent functions of PTEN are not independently responsible for its tumour suppressor functions in many organ systems [21–23]. The frequent loss of PTEN function and the activation of PI3K signalling observed in many, probably most, tumours, has motivated the development of a range of drugs targeting different points within the PI3K signalling network, most notably the PI3Ks themselves, AKT and further downstream, the growth promoting TOR kinase, which is activated in part by AKT [24,25] (Fig. 1). These efforts, involving most of the world's major pharmaceutical companies, have provided a range of drugs that relatively selectively inhibit their targets in the clinic, but although there have been some notable successes [26,27], response rates for these drugs as monotherapies against solid tumours have generally been disappointing [28,29].

2. The PTEN protein and the functional consequences of mutation

2.1. The PTEN protein and its post-translational regulation

The *PTEN* gene encodes a 403 amino acid cytosolic protein [1–3,10], here termed PTEN, and also a recently described 576 amino acid protein which includes a 173 amino acid N-terminal extension. This longer protein is termed PTEN-L or PTEN-Long, and its function is currently unclear: it has been proposed to be secreted and potentially enter other cells or alternatively play a role in mitochondria [30–32]. Almost all functional studies of PTEN have used the originally isolated 403 amino acid protein, although approaches interfering with the function of endogenous PTEN would generally interfere with both PTEN and PTEN-L proteins, making the functional distinctions between the two currently hard to judge. Here we will use 'PTEN' to refer to the 403 amino acid form.

Most of PTEN is made up of an N-terminal phosphatase domain (amino acids 7–185) and a tightly associated C2 domain (186–351) which are required together for protein stability and catalysis [33–35] and also both contain basic residues required for transient membrane association and co-localisation with its phosphoinositide substrate [34,36–38] (Fig. 2). The less tightly structured C-terminal tail of PTEN (352–403) appears to play regulatory roles, containing two clusters of phosphorylation sites. Four phosphorylatable residues in an acidic Ser-Asp-Thr-Thr-Asp-Ser cluster at 380–385 appear to be phosphorylated by CK2 to relatively high stoichiometry in analysed cells and tissues and a further more N-terminal group, commonly displaying lower stoichiometry, encompass phosphorylation at Ser 370, perhaps also by CK2, priming for phosphorylation at Thr 366 and perhaps Ser 362 by GSK3 [39–42]. Phosphorylation at the 380–385 cluster is generally inhibitory to function. A series of consistent studies support a model in which these phosphorylations lead to a closed conformation through interaction of this phosphorylated acidic tail with the basic Phosphatase-C2 core, greatly reduced interaction with membranes and less biological activity and additionally increased protein stability, apparently as a secondary consequence of reduced membrane localisation [38,41–45]. Further sites of phosphorylation including Ser, Thr and Tyr residues have been identified, which appear to be more cell-type selective in their phosphorylation, or at least exhibit lower stoichiometry. Sites have also been identified at which PTEN is oxidised and/or nitrosylated (C71, C124, C83), ubiquitinated (including Lys13, Lys289), SUMOylated (Lys254, Lys266) and acetylated (Lys 125, Lys128, Lys163, Lys402) although in most of these examples of post-translational regulation, a clear picture is yet to emerge of how these modifications of PTEN alter its function and integrate into mechanisms of cellular regulation and this area has been reviewed elsewhere [46–48].

2.2. Experimental analysis of the functional consequences of PTEN mutations

One consequence of the requirement for both the PTEN phosphatase and C2 domains to form a minimal stable catalytic unit is that any truncation or frameshift mutation leads to a complete loss of stability and activity, other than mutations in the sequences encoding the C-terminal tail (amino acids 352–403). In addition, truncating mutations located within the first eight exons lead to mono allelic expression by nonsense mediated decay [49]. Conversely, any mutation in the C-terminal tail encoding by the ninth exon, seems very unlikely to directly disrupt catalytic activity, and more likely instead to influence protein stability and regulation. In light of this, it is notable that although across many tumour types there seems to be an underrepresentation of mutations in sequences encoding the C-terminal tail, a significant number of mutations have been described there from sporadic gliomas (15 C-terminal mutations/734 total mutations in PTEN) and colorectal cancers (10/387, omitting 11 mutations at aa 352 and 5 silent mutations), but never in endometrial cancer (0/978) and very few indeed in any germline cases (2/454) [50–53].

The PTEN protein fulfils a complex array of functions which have been reviewed elsewhere [9–11]. Most notably, it regulates cell growth and proliferation, in part via influences on the AKT group of kinases [54] and also influences processes including chemotaxis and epithelial cell and neuronal polarisation through mechanisms involving localised PTEN activity and downstream mediators [43,55–57]. PTEN displays unusually high protein sequence conservation. For example, the human and murine PTEN proteins have only one conservative Ser-Thr amino acid difference, 99.75% identity, whereas the genomic average human-murine ortholog conservation is around 85% amino acid identity [58]. Recent data from the 1000 Genomes project (www.1000genomes.org) gives us a picture of variation within human populations, revealing very little variation within PTEN. This database lists 54 single nucleotide

Please cite this article in press as: N.R. Leslie, M. Longy, Inherited *PTEN* mutations and the prediction of phenotype, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.01.030