European Journal of Cancer (2014) 50, 216–225

Available at www.sciencedirect.com

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Review

The multiple layers of non-genetic regulation of PTEN tumour suppressor activity

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Available online 19 September 2013

KEYWORDS
PTEN
Tumour suppressor
PI3K–Akt/PKB pathway
Non-genetic inactivation
Epigenetic, transcriptional, post-transcriptional and post-translational regulation
Cancer
Leukaemia

Abstract Mutations and deletions of the tumour suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN) are frequently involved in the development of cancer. However, PTEN is also tightly controlled by various non-genomic mechanisms. This review focuses on those mechanisms, namely on the epigenetic silencing of PTEN, post-transcriptional regulation by non-coding RNAs and post-translational modification. We summarise their involvement in cancer in general, and place some emphasis on leukaemia, where PTEN genetic lesions are relatively uncommon and, strikingly, high levels of PTEN expression frequently associate with PTEN functional inactivation. Overall, it is apparent that rather than looking strictly for PTEN genetic lesions and PTEN expression status, the key to evaluating the real impact of PTEN as a 'quasi-insufficient' tumour suppressor must rely on the complete understanding of PTEN's 'functional dose', incorporating the multiple layers of PTEN regulation in the cell that are ultimately compromised in a given cancer.

1. Introduction

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) also known as mutated in multiple advanced cancers (MMAC), was identified in 1997 by two independent research groups as a candidate tumour suppressor gene that mapped at the human chromosome 10q23, a locus frequently disrupted in primary human cancers [1,2]. In fact, both names summarise important features of the gene.

PTEN belongs to the tyrosine phosphatase family – although PTEN's most prominent role pertains to its lipid phosphatase activity, namely the ability to dephosphorylate the 3-position of the inositol ring in phosphatidylinositol 3,4,5-trisphosphate (PIP3). Since PIP3 is the main cellular product of the class I phosphoinositide 3-kinases (PI3K), PTEN activity constitutes a major negative regulator of PI3K signalling. Loss of PTEN function therefore leads to activation of PI3K downstream signalling, with recruitment of a subset of

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http://dx.doi.org/10.1016/j.ejca.2013.08.017

proteins that contain pleckstrin homology domains to the cell membrane, including the serine/threonine kinases Akt/protein kinase B (PKB) and PDK1. Akt/PKB regulates numerous targets and stimulates cellular processes such as survival, proliferation, metabolism and motility, known to be involved in tumourigenesis [3–5]. PTEN is mainly localised in the cytosol and nucleus, with only a small fraction of cytosolic PTEN being dynamically bound to the plasma membrane under normal conditions [6,7]. Lipid phosphatase-independent roles of PTEN, although poorly characterised, have also been described (reviewed in [8,9]). PTEN is able to dephosphorylate peptide substrates on phospho-serine/threonine and specially phospho-tyrosine residues and it has been proposed that the major role for PTEN protein phosphatase activity is auto-dephosphorylation-mediated regulation of its lipid phosphatase activity [10].

PTEN is a haploinsufficient tumour suppressor, i.e. one functional allele is not sufficient to sustain a wild-type condition. In fact, there is evidence that cells are ultrasensitive to even subtle decreases in PTEN abundance, leading to the inclusion of *PTEN* in the list of 'quasi-insufficient' tumour suppressor genes [11]. In this context, mechanisms that repress *PTEN* expression or result in aberrant subcellular PTEN compartmentalisation are associated with tumourigenesis, and may be much more prevalent and biologically determinant to cancer progression than previously recognised. For example, frequent PI3K pathway hyperactivation in haematological tumours is not paralleled by mutations of PTEN and PI3K [12,13] (reviewed in [14]), suggesting that non-genomic alterations of PTEN may have a profound oncogenic impact.

2. Transcriptional regulation

Disruption of the pathways involved in the regulation of *PTEN* transcription has been thoroughly documented in pathologic conditions (reviewed in [15]). *PTEN* transcription is positively and negatively regulated by different transcription factors (Fig. 1). For instance, the Ras/Raf/MEK/ERK pathway suppresses *PTEN* through c-Jun [16]; and nuclear factor kappa-B (NF-κB) negatively regulates *PTEN* expression through sequestration of the transcriptional coactivator CBP/p300 [17]. Interestingly, the NOTCH1 targets MYC and HES1 were shown to transcriptionally induce and inhibit *PTEN*, respectively [18], although HES1 prevails over MYC in such way that the overall effect of NOTCH1 is to downregulate PTEN [18–20]. In contrast, p53 can upregulate *PTEN*, with a complex interplay between these two tumour suppressors [21,22], and EGR1 can bind to the *PTEN* promoter and upregulate PTEN expression in response to radiation [23] and insulin-like growth factor-2 (IGF2) stimulation [24]. Other transcriptional activators of PTEN expression include PPARγ [25] and the ATF-2, the latter of which is activated by via the p38MAPK pathway [26].

3. Epigenetic silencing

Hypermethylation of CpG dinucleotide-rich regions (CpG islands), and consequent shutting off of tumour suppressor genes, is commonly implicated in cancer [27]. The *PTEN* promoter is hypermethylated in 48% of sporadic breast cancer [28], 16% of hepatocellular carcinoma [29] and more than 50% of thyroid cancers [30]. In acute lymphoblastic leukaemia (ALL) of both B- and T-cell origin, the *PTEN* promoter is hypermethylated in around 20% of the cases, with a higher frequency in adults [31], and *PTEN* promoter hypermethylation was linked to chemoresistance. Philadelphia positive (Ph+) ALL patients are less susceptible to imatinib-induced apoptosis, apparently due to lack of PI3K/Akt pathway inhibition. PTEN downregulation and promoter hypermethylation is observed in 22% of Ph+ ALL patients. The use of a de-methylating agent associates with increased expression of PTEN and cell apoptosis, suggesting that imatinib resistance depends, at least in part, on *PTEN* epigenetic downregulation [32]. Similar findings were reported in chronic myeloid leukaemia [33].

Histone acetylation may also control *PTEN* expression. SALL4, a transcription factor involved in embryonic and leukaemic stem cell self-renewal, was shown to interact with NuRD, a repressor complex with histone deacetylase (HDAC) activity, at the *PTEN* promoter region. This leads to *PTEN* inhibition and potentially explains the development of acute myeloid leukaemia (AML) in SALL4 transgenic mice [34]. Moreover, the transcription factor Evi1 is ectopically expressed in approximately 10% of AML patients. Evi1 binds to polycomb group proteins that catalyse the addition of a methyl group at Lys27 of histone H3 (H3K27me), promoting silencing of transcription through chromatin modification. Evi1 recruits polycomb complexes to the *PTEN* locus and activates the Akt/mammalian target of rapamycin (mTOR) signalling pathway through transcriptional repression of *PTEN* [35], functioning as a bridge between the epigenetic machinery and signalling pathway activation.

4. Post-transcriptional regulation

MicroRNAs represent a further level of regulation of PTEN, contributing to PTEN decreased expression in specific cancers (Fig. 1). For instance, the oncogenic miR-21 directly targets *PTEN* in hepatocellular carcinoma [36]; it is overexpressed in human ovarian carcinoma, likely impacting PTEN [37]; and downregulates PTEN and stimulates growth and invasion in non-small cell lung cancer [38]. Interestingly, miR-21 targets also