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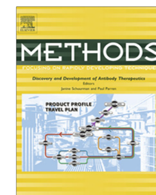

Analysis of PTEN ubiquitylation and SUMOylation using molecular traps

Valérie Lang [a], Fabienne Aillet [a], Elisa Da Silva-Ferrada [a], Wendy Xolalpa [a], Lorea Zabaleta [a], Carmen Rivas [b,c], Manuel S. Rodriguez [a,*]

[a] Inbiomed, Ubiquitylation and Cancer Molecular Biology Laboratory, Cancer Unit Mikeletegi 81, 20009 San Sebastián-Donostia, Spain

[b] Dpt Biología Molecular y Celular, Centro Nacional de Biotecnología-CSIC, Darwin 3, Madrid 28049, Spain

[c] Centro de Investigación en Medicina Molecular y Enfermedades Crónicas (CIMUS), Universidad de Santiago de Compostela, Instituto de Investigaciones Sanitarias (IDIS), Santiago de Compostela E15706, Spain

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ABSTRACT

The function of the tumour suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is tightly controlled by post-translational modifications (PTMs) including ubiquitin or Small Ubiquitin-related MOdifiers (SUMO). It is known that SUMOylation by SUMO-1, SUMO-2/-3, mono- or polyubiquitylation have a distinct impact on PTEN activity, localisation and/or stability, however the molecular mechanisms governing these processes are still unclear. Studying PTM regulated events has always been a difficult task due to their labile nature. Here, we propose an update on the role of these PTMs on PTEN function, as well as a methodological overview on the use of molecular traps named SUMO Binding Entities (SUBEs) or Tandem Ubiquitin Binding Entities (TUBEs) to capture SUMOylated or Ubiquitylated forms of PTEN respectively. When combined with *in vitro* SUMOylation or Ubiquitylation assays, the use of molecular traps facilitate the detection of modified forms of PTEN. SUMO and ubiquitin-traps are also suitable to capture endogenously modified forms of PTEN after expression of E3 ligases or treatment with chemical inhibitors. This versatile approach represents an interesting alternative to explore PTEN regulation by SUMO and ubiquitin under physiological or pathological conditions.

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1. Introduction

Protein modification by members of the ubiquitin family is among one of the most investigated mechanisms of cellular regulation. Ubiquitin and ubiquitin-like (UbL) molecules such as SUMO-1, SUMO-2 and SUMO-3 are attached to protein substrates through similar biochemical processes that implicate distinct sets of enzymes [1,2]. The attachment (or conjugation) of ubiquitin or UbL modifiers is mediated by a thiol–ester cascade of reactions that requires the action of 3 enzymes: an activating enzyme or E1 that activates molecules required for the different reactions and a conjugating enzyme or E2 that frequently conjugates protein modifiers to their targets. The action of ubiquitin ligases or E3s is required to recognise target proteins, facilitating their ubiquitylation [1,2]. While ubiquitin can potentially modify any lysine residue in a protein substrate, SUMO molecules are frequently attached within the consensus sequence ψ-K-X-D/E (where ψ is a hydrophobic amino acid and X is any amino acid) [3]. In *in vitro* assays, where E2s are abundant, protein ubiquitylation or SUMOylation can be achieved even in the absence of E3s [4] but it is unclear if this can occur at physiological levels.

Different chain types have been associated to distinct functions. Ubiquitin chain linkages K48 and K11 have been related to proteolysis mediated by the proteasome, whereas K63 chains appear to direct signalling events, endocytic trafficking or DNA repair. Attachment of SUMO-1 or SUMO-2/-3 has different physiological outputs sometimes counteracting or contributing to ubiquitin conjugation [5–9]. SUMO and ubiquitin can also act sequentially in the regulation of the promyelocytic leukaemia protein (PML). The conjugation of SUMO-2/-3 chains on PML allows the recruitment of the SUMO-binding ubiquitin ligase RNF4 (ring finger protein 4), resulting in the targeting of ubiquitylated PML to proteasome-mediated degradation [10–12]. The formation of heterologous chains containing ubiquitin and SUMO-2/-3 has also been reported for other substrates [13–15] but a generic function of these chains

Abbreviations: TUBEs, Tandem Ubiquitin Binding Entities; SUBEs, SUMO Binding Entities; PTMs, post-translational modifications.

* Corresponding author. Fax: +34 943308222.

E-mail addresses: vlang@inbiomed.org (V. Lang), faillet@inbiomed.org (F. Aillet), edasilva@inbiomed.org (E. Da Silva-Ferrada), wendyxolalpa@gmail.com (W. Xolalpa), lzabaleta@inbiomed.org (L. Zabaleta), mcarmen.rivas@usc.es (C. Rivas), msrodriguez@inbiomed.org (M.S. Rodriguez).

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has not been yet established. De-ubiquitylating enzymes or DUBs recognise and cleave specific ubiquitin chains from target substrates through ubiquitin binding domains (UBDs) [16,17]. An equivalent reaction is achieved by SUMO-specific proteases (SENPs) [18].

Since PTEN is involved in several cellular processes, its function is tightly regulated by multiple post-translational modifications, including acetylation, phosphorylation, SUMOylation as well as mono- and polyubiquitylation [19–25]. Ubiquitylation plays one of the most significant regulatory roles on PTEN by controlling its degradation and compartmentalisation [25,26]. A lysine mutant of PTEN, K289E associated with Cowden syndrome, retains catalytic activity but fails to accumulate in nuclei of patient tissue due to a nuclear import defect [25]. K289 and K13 are the major monoubiquitination sites essential for PTEN nuclear import [25]. While nuclear PTEN is stable, polyubiquitination leads to its degradation in the cytoplasm. Several ubiquitin ligases appear to act on PTEN. The E3 ubiquitin ligase NEDD4-1 directly interacts with PTEN and its overexpression promotes PTEN mono- and polyubiquitylation depending on p34 [26,27]. While PTEN polyubiquitylation leads to a classical proteasome-mediated degradation, monoubiquitylation regulates its nuclear import [26] (Fig. 1). PTEN C-terminal tail plays a role in the regulation of PTEN stability since its deletion increases the binding to NEDD4-1 and consequently its ubiquitylation. [28]. Other ubiquitin E3s have been reported, including the X-linked inhibitor of apoptosis (XIAP), WW-domain-containing protein 2 (WWP2) and the C terminus of Hsc70-interacting protein (CHIP) [29–31]. The fact that multiple ubiquitin ligases act on PTEN underlines the critical role of this tumour suppressor and thus its tight regulation [32–37].

PTEN has also been reported to be SUMOylated at both K266 and K289 sites [7,38]. SUMO-1 modification of K266 is mainly responsible for PTEN association with the plasma membrane and the inhibition of PI3K–AKT signalling pathway [7] (Fig. 1). More recently the SUMOylation of PTEN K254 has been associated to DNA repair and genotoxic stress [39]. A crosstalk between PTEN SUMOylation and ubiquitylation has also been proposed. Indeed PTEN-SUMO-1 shows a reduced capacity to form covalent interactions with monoubiquitin [38]. As it occurs for proteins such as IκBα or PCNA, a potential competition of both modifications acting on the same lysine residue could explain this phenomenon [5,40,41]. On the other hand the accumulation of PTEN-SUMO-2 is observed after inhibition of the proteasome underlining the participation of SUMO-2 in a proteasome dependent proteolysis of PTEN [38] (Fig. 1). The role of PIAS (protein inhibitor of activated STAT) proteins on PTEN modification has been recently described. From the five PIAS family members (PIAS1, PIAS3, PIASxα, PIASxβ and PIASy), PIASxα shows physical interaction with PTEN and promotes its efficient modification [28]. Coherently, PIASxα enhanced PTEN protein stability by reducing PTEN ubiquitylation and promoting its tumour-suppressive functions [28]. Other members of the PIAS family appear to induce, in a minor proportion, PTEN SUMOylation. However, their physiological role is yet to be established.

To better understand the role of ubiquitin and SUMO in the regulation of PTEN, it is important to consider both modifications on the same cellular context. For instance, inhibition of PTEN ubiquitylation negatively affects axonal branching in retinal ganglion cells whereas the silencing of PTEN rescues this process [32]. In other words, it seems that the key step is the ubiquitylation and not the proteolysis. Similarly, PTEN ubiquitylation is essential to regulate PI3K–AKT signalling and neuronal survival after treatment with Zn^{2+} [34]. Most of these processes require the participation of interacting factors that modulate the recognition of PTEN by

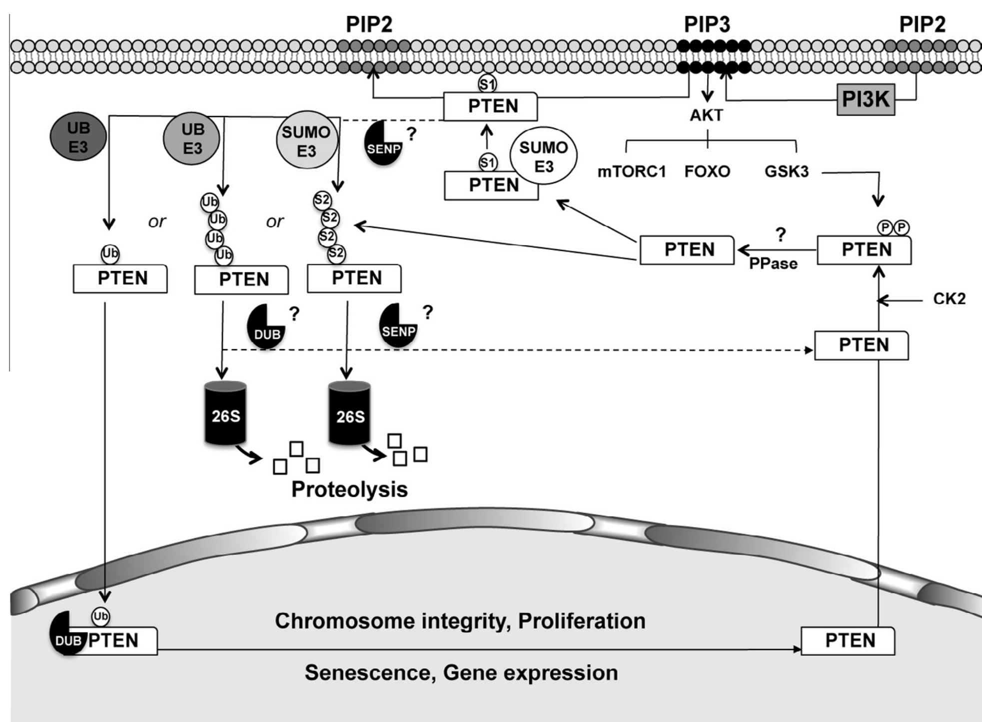

Fig. 1. Integrated view of the role of ubiquitin and SUMO molecules on the control of PTEN localisation, stability and function. This hypothetical model assumes that PTEN conjugated to SUMO-1 is recruited to the cellular membrane to exert its phosphatase activity. PTEN can be also regulated by SUMO-2 or ubiquitin through mechanisms that include changes in stability and subcellular localisation. Rescuing PTEN from degradation would somehow explain its well-known stability in many cell types. In this model, the action of unidentified (?) SUMO-specific proteases (SENPs) or de-ubiquitylating enzymes (DUBs) would be required at different steps. The only identified DUB for PTEN, USP7 plays a role in the nucleus and allows the relocalisation of PTEN in the cytoplasm [53]. While several ubiquitin ligases (see main text) have been implicated in the modification of PTEN, only one SUMO ligase, PIASxα has been reported until now. Another way to regulate PTEN stability and activity is by increasing the proportion of phosphorylated forms mediated by several kinases.

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