Importin-11 keeps PTEN safe from harm

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In this issue, Chen et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201604025) show that Importin-11 traffics the tumor suppressor PTEN into the nucleus and in so doing protects it from cytoplasmic proteins that cause PTEN degradation. This work helps explain the nuclear accumulation of PTEN observed in many healthy tissues and, because *Ipo11* mutant mice develop lung tumors, also implicates Importin-11 as a novel tumor suppressor.

It has been clear for some time that the tumor suppressor PTEN shuttles in and out of the nucleus. Furthermore, many lines of evidence imply that this nuclear localization has a big influence on the activities of PTEN, which control cell behavior and block tumor formation. However, detailed understanding of how PTEN gets in and out of the nucleus and how this affects PTEN function has been slow to develop.

The first well-controlled studies of PTEN expression and localization in tissue samples from cancer patients provided a consistent but puzzling picture. In several normal tissues, PTEN appeared to be largely nuclear, but in tumors PTEN was either absent or largely observed in the cytosol (Gimm et al., 2000; Perren et al., 2000; Tachibana et al., 2002). A picture steadily emerged that nuclear PTEN was a good thing. However, simultaneously strong evidence established that PTEN is a lipid phosphatase that acts on a substrate almost exclusively located within the plasma membrane, phosphatidylinositol 3,4,5-trisphosphate (PIP_3; Worby and Dixon, 2014). It also became clear that PIP_3 metabolism is the dominant mechanism of tumor suppression by PTEN. To address this conundrum, several studies have proposed that PTEN fulfils novel tumor suppressor functions in the nucleus unrelated to its PIP_3 phosphatase activity (Worby and Dixon, 2014). However, the finding by two independent research groups that mice expressing mutant PTEN proteins that selectively lack lipid phosphatase activity develop worse tumors than mice expressing no PTEN argues strongly against PTEN having dominant tumor suppressor functions that act fully independently of its PIP_3 phosphatase activity. This leaves the question of why nuclear PTEN correlates with tumor suppression.

Like many proteins, the function of PTEN is controlled in part by ubiquitination—the covalent ligation of units of the small regulatory protein ubiquitin. The addition of a single ubiquitin unit to PTEN seems to promote a nuclear localization for PTEN. In contrast, as seen with many proteins, the addition of multiple linked ubiquitin units targets PTEN for destruction. Further pieces of this complex puzzle have been provided. Several lysine residues on PTEN have been identified that can carry ubiquitin (Trotman et al., 2007; Gupta and Leslie, 2016). Several ubiquitin E3 ligases have been shown to couple ubiquitin onto PTEN (Wang et al., 2007; Van Themsche et al., 2009; Maddika et al., 2011; Ahmed et al., 2012; Lee et al., 2013, 2015; Li et al., 2014) and conversely enzymes that remove ubiquitin from PTEN have been identified. However, details of how these enzymes influence PTEN function, where in cells they act, and how these ubiquitin chain linkages are extended and at which of the sites on PTEN has been largely unknown.

In new research published in this issue, Chen et al. provide strong evidence that the importin β family member Importin-11 (IPO11) binds to PTEN and transports it into the nucleus. In prostate cancer cells lacking IPO11 function, PTEN was found to be much more cytoplasmic at steady state than control cells and in dynamic experiments IPO11 disruption greatly slowed down entry of PTEN into the nucleus. Importantly, if IPO11 was stopped from taking PTEN into the nucleus, not only did this stop accumulation of PTEN in the nucleus but also greatly reduced the level of PTEN protein expression without affecting the abundance of its mRNA. These data are consistent with the idea that PTEN may be degraded in the cytoplasm and fits with proposed roles of the HECT domain containing E3 ubiquitin ligase NEDD4 and its binding partner NDFIP1 in promoting PTEN degradation and with their expected cellular locations. However, hints that disruption of IPO11 function reduced PTEN levels far more strongly than simply excluding PTEN itself from the nucleus motivated the authors to investigate other recognized IPO11 cargo proteins that might contribute to the regulated destruction of PTEN. When tested, one such IPO11 cargo, the E2 ubiquitin–conjugating enzyme UBE2E1, was shown to drive PTEN ubiquitination in cells when overexpressed and to be required for the degradation of PTEN caused by loss of IPO11.

This led Chen et al. (2017) to propose a "relay break" model in which PTEN is monoubiquitinated on Lys13 and/or Lys289 promoting binding to IPO11, which in turn traffics both this monoubiquitinated PTEN and UBE2E1 into the nucleus (Fig. 1). This separates PTEN from the cytoplasmic E3 ubiquitin ligases such as NEDD4 and its Golgi membrane–located binding partner NDFIP1, which would drive PTEN polyubiquitination and degradation. It also separates the E2 component UBE2E1 from the E3 NEDD4–NDFIP complex. It is recognized that such a model is affected by PTEN nuclear export and deubiquitination, which are less clearly understood.

To investigate whether IPO11 affected the functions of PTEN as a tumor suppressor, *Ipo11* mutant hypomorphic mice that carry an exogenous splice acceptor site in the *Ipo11* gene

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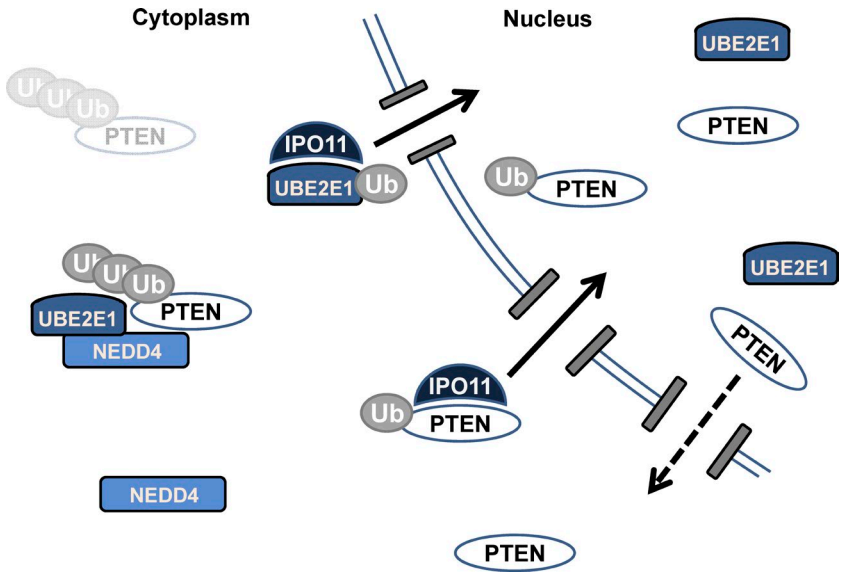

Figure 1. **PTEN nuclear import and ubiquitination.** Chen et al. (2017) propose a "relay break" model for the regulation of PTEN degradation (see Fig. 4 E in Chen et al., 2017). PTEN, which is monoubiquitinated (Ub) in the cytosol and primed for further polyubiquitination, marking it for degradation, is also a substrate for import into the nucleus by IPO11. One effect of nuclear import is to protect PTEN from cytosolic degradation initiated by E3 ubiquitin ligase components, including NEDD4. IPO11 also traffics the activated UBE2E1 E2 ubiquitin–conjugating enzyme into the nucleus, separating it from cytosolic enzymes that could combine with UBE2E1 to promote the polyubiquitination of PTEN. PTEN deubiquitination and nuclear export are recognized but how these actions complement IPO11-driven nuclear import is unclear.

to reduce its expression level were bred. Homozygous *Ipo11* hypomorphs are born at lower frequencies than expected, indicating some embryonic lethality. In addition, whereas in some tissues and organs (e.g., mouse embryo fibroblasts and lung tissue) IPO11 expression was greatly reduced, in others, such as excised prostates, IPO11 expression appeared normal. Lung tumors were observed in the *Ipo11* mutant mice and their tumor-free survival was significantly worse than a control cohort. These lung tumors, mostly adenomas and adenocarcinomas, showed low staining for IPO11 and PTEN and weak diffuse UBE2E3 expression, indicating little IPO11 function. The tumors also showed high levels of P-AKT and Ki67 staining, indicating enhanced growth signaling and cell proliferation, respectively. Although it is clearly possible that these lung tumors are driven by other factors such as disturbances in the function of other IPO11 cargo proteins, the data do suggest that IPO11 acts as a tumor suppressor at least in part by trafficking and protecting PTEN.

Following on from these mouse studies, Chen et al. (2017) looked at human clinical data for evidence of loss of IPO11 function in human cancers as would be predicted if it acts as a tumor suppressor. This revealed a highly significant correlation between reduced levels of IPO11 and reduced levels of PTEN seen in approximately half of the large cohort of lung cancer tissue samples. However, no evidence for loss of expression at the mRNA level was seen, consistent with the idea that IPO11 acts directly on the PTEN protein. Correlations were also observed in prostate cancer between IPO11 loss and poor clinical outcomes and progressive metastatic disease.

Of course new questions are raised by this work and old ones remain regarding many aspects of PTEN ubiquitination and regulation. For example, does PTEN need other factors and inputs to stop its cytosolic destruction if it is to accumulate there and access PIP_3 in the plasma membrane? Also, it is still unclear where and how the many different proposed E3 ubiquitin ligases and ubiquitin proteases act on PTEN and even less clear whether blocking PTEN degradation represents a viable target for therapeutic intervention. However, the study by Chen et al. (2017) provides some important new pieces in the complex PTEN regulatory jigsaw puzzle. It also identifies IPO11 as a possible new tumor suppressor. Loss of one IPO11 gene copy has been observed at high frequencies (>30%) in many cancer genomic datasets, including lung cancer studies, and although other neighboring genes may contribute to driving these deletions (including, e.g., the PIK3R1 gene), the in vivo tumorigenesis data of Chen et al. (2017) argues strongly that IPO11 is likely to be individually important as a tumor suppressor in many of these cases. The observation that only some tissues (e.g., lung) in the authors' IPO11 mutant mouse had substantial reductions in IPO11 expression leaves open the possibility that the IPO11–PTEN axis may be important in many more cancer types and seems likely to be the subject of further study.

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