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SPOTLIGHT

A balancing act: PHLPP2 fine tunes AKT activity and MYC stability in prostate cancer

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PTEN loss stimulates prostate tumor progression by sustaining AKT activation. Nowak et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201902048) surprisingly show that the AKT-suppressing phosphatase PHLPP2 promotes disease progression in the context of dual PTEN and p53 loss by increasing MYC stability.

Advanced, metastatic prostate cancer (PC) is commonly characterized by loss of expression of the tumor suppressor PTEN, often in combination with p53 inactivation (1). Sustained AKT activation in the absence of PTEN can lead to PC initiation and progression (2, 3). This has led to clinical trials focusing on inhibition of MTOR in PC, with disappointing results (4). To study the role of these tumor suppressors in disease progression, the Trotman group previously developed the RapidCaP mouse model (5) in which knockout of floxed genes is performed by injection of a CRE-expressing virus directly into the prostate. Knockout of PTEN and p53 led to metastatic disease, but, surprisingly, the metastases had lower AKT activation than the primary tumor (5), which a later study found was driven by up-regulation of the Akt-supressing phosphatase PHLPP2 (6). Analysis of signaling and oncogenes expressed in these metastases also revealed up-regulation of MYC expression. In this issue, Nowak et al. further investigate the mechanistic role of PHLPP2 in AKT suppression and MYC activation.

Using Cre-infected *Pten;p53* floxed mouse embryonic fibroblasts (MEFs), Nowak and colleagues (7) first demonstrate that combined loss of p53 and PTEN leads to an increase in PHLPP2 phosphatase expression and increased MYC abundance. Genetic ablation of *Phlpp2* in these MEFs led to a strong decrease in MYC protein and reduced cell proliferation. They validated this inverse relationship between PHLPP2 expression and MYC stability in MEF, DU145, and PC3 cells using CRISPR, siRNA, and overexpression studies.

To demonstrate the direct interaction between PHLPP2 and MYC and the in vitro capacity of the PHLPP2 phosphatase domain to dephosphorylate MYC, the authors used cell extracts and recombinant proteins. After demonstration that PHLPP2 is coimmunoprecipitated with MYC, they tested the activity of the phosphatase domain (PP2C) of PHLPP2 in dephosphorylating MYC at T58. Interestingly, only the phosphatase domain of PHLPP2, but not the closely related PHLPP1 phosphatase, significantly decreased in vitro phosphorylation of MYC at T58. These data suggest that even though PHLPP1 can inhibit AKT activity in PC, it is unlikely to account for changes in MYC stability, as opposed to PHLPP2 that can dephosphorylate and regulate both proteins.

Using a small molecule inhibitor of PHLPP2, the researchers phenocopied the results obtained by genetic ablation of *Phlpp2*, namely, decreased stability of MYC and increased AKT phosphorylation. The inhibitor demonstrated a reduction in cell viability and proliferation in multiple cell lines. It is noteworthy that although most experiments have been performed in PTEN-null cells, the same results were observed with the inhibitor of PHLPP2 in DU145 cells, which are PTEN positive. This could support the generalization of the usefulness of PHLPP2 inhibition independently of PTEN status.

Finally, to validate their findings in vivo, Nowak et al. (7) used the RapidCaP model to compare disease progression in mice with combined PTEN/p53-deficient prostate with or without PHLPP2. The results showed that localized disease progression is similar in the presence or absence of PHLPP2, but that metastases were not observed in the PHLPP2-deficient setting.

This study highlights an important feature of PC that is often overlooked—MYC post-transcriptional regulation can have a drastic impact on MYC protein abundance and copy number analysis can miss subsets of PC with elevated MYC at the protein level. PIM kinases, which are overexpressed in PC, also stabilize MYC by impacting MYC phosphorylation (8). Investigating the relationship between PIM and PHLPP2 expression in PC could be important to understand the overall stability of MYC. Interestingly, Hubbard et al. (9) have reported that MYC overexpression and PTEN loss leads to distant metastasis without the required inactivation or loss of p53. It is tempting to speculate that this model could maintain higher AKT activity if PHLPP1/PHLPP2 are not overexpressed in a p53-intact setting (9).

Another interesting point raised by Nowak et al. (7) is the possibility of targeting PHLPP2 in prostate tumors that are intact for PTEN but p53 inactivated. Conversely, what would happen to PHLPP2 expression when only one allele of PTEN is lost, in which case the senescence response of PTEN deletion is less pronounced (10) and pressure to lose p53 to overcome senescence may be lower? Although future work is required to establish the biology

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of PHLPP2 expression in tumors driven by other oncogenic insults than combined loss of PTEN and p53, Nowak et al. (7) present evidence for a promising novel therapeutic target for PC.

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