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RESEARCH HIGHLIGHT

"PEST control": regulation of molecular barcodes by tyrosine phosphatases

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The emerging concept of "molecular barcodes" refers to the dynamic combination of post-translational modifications, often of different nature (e.g., phosphorylation and ubiquitination) that gives rise to multiple forms of a protein which can relay distinct signals throughout a cell. In a recent *Cell Research* paper by Wang *et al*., the authors report that a PEST domain-containing tyrosine phosphatase, PTPN18, is able to regulate both phosphorylation and ubiquitination of the HER2 oncogene, barcoding HER2 for increased proteasomal degradation rather than for intracellular trafficking.

HER2 is a prominent oncogene overexpressed in breast cancer and several other solid cancers, and is a drug target of Trastuzumab, a monoclonal antibody that is FDA-approved for treatment of HER2+ breast cancers [1]. HER2 undergoes phosphorylation on several tyrosine residues, each one regulating specific intracellular functions. In a recent paper published in *Cell Research*, Wang *et al*. [2] first defined which phosphorylation sites on HER2 are regulated by protein tyrosine phosphatase non-receptor type 18 (PTPN18). They identified that three HER2 C-terminal tyrosines are dephosphorylated by PTPN18: Y^{1112}, Y^{1196} and Y^{1248}. Previous reports have shown that phosphorylation of Y^{1112} leads to enhanced Cbl-mediated K63-linked ubiquitination and lysosomal targeting of HER2 [3], while Y^{1196} phosphorylation leads to increased cell motility and decreased apoptosis [4, 5], and Y^{1248} phosphorylation activates MAPK signaling [6]. In an elegant series of experiments, and through co-crystallization of PTPN18 with peptides derived from phosphorylated HER2, Wang *et al*. identified "selective loss-of-function" point mutations of PTPN18 that abolish the ability of the phosphatase to dephosphorylate each one of the three above-mentioned HER2 phosphorylation sites. The authors then proceeded to express these selective loss-of-function PTPN18 mutants in breast cancer cells in order to assess the cellular phenotypes elicited by hyperphosphorylation of each PTPN18-target site. When they expressed the PTPN18 P109A mutant, which is unable to dephosphorylate the pY^{1112} site, the authors indeed observed an increase in phosphorylation-dependent HER2 lysosomal trafficking. However, when they subjected cells to complete knockdown of PTPN18, there was a paradoxical increase in HER2 expression. By following this unexpected observation, they identified a new mechanism of PTPN18-mediated regulation of HER2 half-life. They showed that PTPN18 promotes HER2 ubiquitination and proteasomal degradation through a physical interaction between the PTPN18 PEST domain and HER2, which enables the recruitment of the E3 ubiquitin ligase β-Trcp to the HER2 complex. The authors also provided indirect evidence suggesting that phosphorylation of serine residues in the PEST domain of PTPN18 is necessary to promote ubiquitination and degradation of HER2.

In addition to unraveling a new phosphorylation- and ubiquitination-based barcoding mechanism of regulation of the intracellular fate of an important oncogene, this study also introduces two novel concepts in the biochemistry and cellular biology of tyrosine phosphatases. First, by providing an example of structure-based design of mutants able to selectively abolish the capacity of dephosphorylating single sites, this study provides rigorous support to the now widely-accepted notion that phosphatases are not promiscuous enzymes. Rather, they recognize different sites in unique ways that depend on structural determinants outside the active site. While this concept is not new, and has led to the successful design of bi-dentate and multi-dentate tyrosine phosphatase inhibitors with enhanced enzyme selectivity features [7], it is the first time that it is extended to selective targeting of phosphorylation sites within the same molecule. The Wang *et al*. approach to phosphatase selectivity through structural biology paves the way to the rational design of compounds for selective inhibition of single site dephosphorylation, which would be desirable for biology studies and signaling therapies [8].

The second innovative aspect of the report by Wang *et al*. [2] is the demonstration that two domains of a tyrosine phosphatase mediate different post-translational modifications of the same target molecule. Tyrosine phosphatases such as PTPN11 (SHP-2) can regulate signaling transduction through catalytic-dependent and -independent manners [9]. We also recently reported

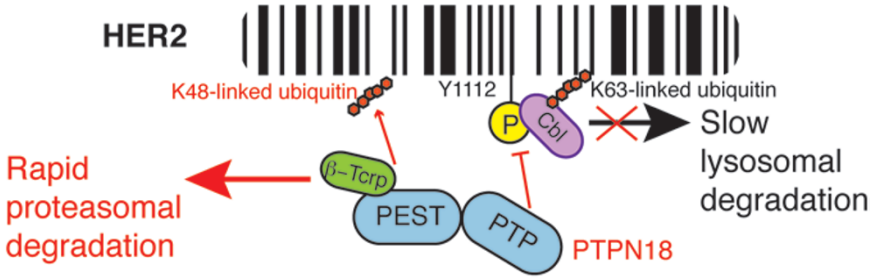

Figure 1 PTPN18 regulates the tyrosine phosphorylation and ubiquitination barcodes of HER2 at specific residues through its catalytic (PTP) and PEST domains.

that another PEST-containing tyrosine phosphatase, PTPN22, promotes the ubiquitination of TRAF3 in innate immune receptor signaling through a catalytic activity-independent mechanism [10]. The Wang *et al*. study goes one step forward by showing that PTPN18 can modify phosphorylation and ubiquitination of the same substrate. The phosphorylation-dependent and the ubiquitination-dependent components of HER2 barcoding are respectively regulated by the catalytic domain and the PEST domain of PTPN18. This suggests that one additional feature conferred to tyrosine phosphatases by their characteristic multi-domain organization [11] is the ability to modify the same substrate using two different mechanisms.

In summary, Wang *et al*. [2] present a study of significant scientific breadth on phosphorylation- and ubiquitination-based barcoding of the oncogene HER2 by the tyrosine phosphatase, PTPN18 (Figure 1). A few questions about the molecular biology of this process remain open and warrant further investigation. For example, it remains to be determined what is the binding site on HER2 that interacts with the PEST domain of PTPN18 and the composition of the HER2 complex that enables ubiquitination and proteasomal targeting of HER2. Further investigations are also needed to clarify whether dephosphorylation or other post-translational modifications of the PEST domain of PTPN18 can alter its function, resulting in different barcoding of HER2 and increased HER2 half-life promoted by the catalytic domain of PTPN18. Further investigation of barcoding of HER2 and other signaling molecules by tyrosine phosphatases would be of high relevance for cancer biology and will improve our understanding of tyrosine phosphatases and the ability to selectively target these enzymes for therapy of human diseases.

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