

IN THE SPOTLIGHT

On the Road to Combinations of Targeted Therapies: PPM1H Phosphatase as a Suppressor of Trastuzumab Resistance

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Summary: Lee-Hoeflich and colleagues use RNA interference screening to identify the serine/threonine phosphatase PPM1H as an inhibitor of trastuzumab resistance *in vitro*. This finding extends the molecular portrait of trastuzumab-resistant cells and provides a rationale when searching for potential therapeutic targets among regulators of PPM1H and/or its substrates. *Cancer Discovery*; 1(4): 285-6. ©2011 AACR.

Commentary on Lee-Hoeflich et al., p. 326(10).

Approximately 20% of human breast cancers overexpress the epidermal growth factor receptor 2 (EGFR, also known as HER2, ErbB2), one of a family of four receptor tyrosine kinases that also includes EGFR/HER1, HER3, and HER4 (1). The relevance of HER2 in cancer is illustrated by the clinical success of the anti-HER2 humanized monoclonal antibody trastuzumab (Herceptin), particularly when used in combination with chemotherapy. Trastuzumab is one of the first examples of a successful targeted therapy and the first monoclonal antibody shown to prolong life in patients with cancer (2-4). Trastuzumab binds to the juxtamembrane portion of the extracellular domain of HER2, and several mechanisms have been proposed to contribute to its action *in vivo*: Trastuzumab disrupts ligand-independent HER2/HER3 complexes, increases HER2 degradation, inhibits the activating cleavage of its extracellular domain, and induces antibody-dependent cell cytotoxicity (5-8).

Despite the clinical efficacy of trastuzumab, not all HER2-positive tumors respond to therapy, and response rates vary, depending on the combination of chemotherapeutic drugs used with trastuzumab (4). Unfortunately, trastuzumab resistance remains an important clinical problem. Proposed mechanisms of resistance to trastuzumab (Table 1) include the activation of the phosphoinositide 3-kinase (PI3K)/Akt/mTOR pathway, for example, via loss of the tumor suppressor lipid phosphatase PTEN (9) and/or activating mutations of *PIK3CA*, the alpha-catalytic subunit of PI3K (5). A clearer understanding of the molecular portrait of trastuzumab-resistant breast cancers should reveal new targets for combination therapy and ultimately lead to more effective treatments.

Lee-Hoeflich and colleagues (10) sought to identify novel mediators of trastuzumab resistance in HER2-positive cancers. They used an siRNA library targeting human kinases and phosphatases with the ultimate goal of identifying genes

whose knockdown affects proliferation during a short-term (72 hours) treatment with trastuzumab *in vitro*. In addition to the well-known suppressors of trastuzumab resistance, PTEN and p27, knockdown of the serine/threonine phosphatase PPM1H increased proliferation in the presence of trastuzumab in monolayer cultures and augmented acinar size in a 3-dimensional culture assay. These data lead to the conclusion that PPM1H may act as a suppressor of trastuzumab resistance and raise the possibility that low levels of PPM1H may facilitate proliferation of trastuzumab-treated cells *in vivo*. Further experiments using *in vivo* models of breast cancer are needed to determine whether this phosphatase really is a predictive marker of resistance to trastuzumab.

How does the expression of PPM1H in tumors affect the survival of breast cancer patients? The authors examined 87 HER2-positive primary tumor samples from patients treated with trastuzumab and found a trend between low PPM1H expression in patients (detected with an isotopic *in situ* hybridization probe) and poor clinical outcome. This observation warrants further study with a larger cohort of patients to assess its significance. Ultimately, analysis of PPM1H expression in matched biopsies from patients before and after treatment with trastuzumab might reinforce the clinical relevance of these findings.

Table 1. Proposed mechanisms of trastuzumab resistance

Mechanism of trastuzumab resistance	Reference
PTEN loss	Nagata et al. (9)
PIK3CA activating mutations	Junttila et al. (5)
p27 downregulation	Nahta et al. (13)
Src activation	Zhang et al. (15)
EGFR and ErbB ligands overexpression	Ritter et al. (16)
Met activation	Shattuck et al. (17)
IGF1R activation	Nahta et al. (18)
P95HER2 expression	Scaltriti et al. (19)
Cyclin E amplification/overexpression	Scaltriti et al. (20)
MUC4 overexpression	Nagy et al. (21)

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To date, very little is known about the roles of the serine/threonine phosphatase PPM1H in cancer. Previously, PPM1H was found to be upregulated in colon adenocarcinomas compared with normal colon tissues (11). However, no attempt was made to examine the roles of this phosphatase in breast cancer or in drug resistance. Furthermore, the mechanism of action of PPM1H remained unclear. After discovering PPM1H as a potential suppressor of trastuzumab resistance, Lee-Hoeflich and colleagues (10) investigated its molecular mechanism of action and found that knockdown of PPM1H consistently reduced protein levels of the cell-cycle regulator and well-known tumor suppressor p27. Loss of p27 protein has indeed been strongly associated with high histopathologic tumor grade and poor patient outcome (12). In addition, loss of p27 was found previously to be associated with trastuzumab resistance in breast cancer cells (13). Lee-Hoeflich et al. have shown that PPM1H dephosphorylates p27 at threonine 187, removing a signal for proteosomal degradation. Thus, loss of PPM1H triggers increased phosphorylation and degradation of p27, which leads to increased cell proliferation. Further experiments, including unbiased phosphoproteomic analysis, may show that loss of PPM1H results in the hyperphosphorylation of proteins other than p27.

The initial positive response to most targeted cancer therapies is often thwarted by development of resistance by cancer cells. Therefore, identification of resistance mechanisms is of paramount importance for the rational design of combination therapy. In the last decade, key resistance mechanisms to trastuzumab treatment of breast cancers were described (Table 1), paving the way for the use of combinations of targeted therapies—for example, the combination of PI3K inhibition and trastuzumab that is currently in clinical trials. An urgent question now is whether further combinations should be prioritized and in which sequence therapies should be administered. Answers to these questions will require serial monitoring of tumor evolution upon administration of targeted therapy, to rationally design clinically effective regimens. An elegant example of such an approach was recently reported for non-small cell lung cancers with *EGFR* mutations (14). Although the study by Lee-Hoeflich et al. (10) did not identify a therapeutic target, it has provided a potential predictive marker of resistance to trastuzumab and a rationale for searching for targets among upstream regulators of PPM1H and/or its downstream substrates. This contribution is an important initial step on the road to identifying targets that could be inhibited in combination with trastuzumab.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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