Successful targeting of ErbB2 receptors—is PTEN the key?

Women with ErbB2-positive breast cancer have a poor prognosis, and frequently, chemotherapy treatment is ineffective. The ErbB2-targeted antibody trastuzumab improves survival when given with chemotherapy to patients with ErbB2-overexpressing metastatic disease, but treatment is not curative, and primary resistance is common. Postulated mechanisms of action for trastuzumab include immune-mediated cytotoxicity and receptor downmodulation. A study in this issue of *Cancer Cell* suggests that trastuzumab causes rapid activation of the PTEN lipid phosphatase, which in turn downregulates the phosphatidylinositol 3′-kinase (PI3K) pathway. Resistance to trastuzumab occurs when PTEN function is lost, suggesting that PTEN activation is a critical component of the therapeutic effect.

Trastuzumab, a humanized monoclonal antibody, is an effective treatment for advanced breast cancer with *ErbB2* gene amplification. While not a cure for disseminated disease, major tumor regressions are often seen, particular in combination with chemotherapy. Despite careful patient selection on the basis of *ErbB2* fluorescence in situ hybridization, only a minority of patients respond to trastuzumab monotherapy. Furthermore, trastuzumab has not shown as much efficacy against other ErbB2-expressing malignancies such as lung and pancreas cancer. Overall, these observations suggest that *ErbB2* gene amplification is necessary but not sufficient for trastuzumab responsiveness.

What are the other factors that determine trastuzumab efficacy? An answer to this question could provide new predictive biomarkers, as well as strategies to improve efficacy. Insights are limited, mostly because it is not clear how trastuzumab actually works. The antineoplastic effect might depend on engaging Fc γ receptors on immune effector cells through the immunoglobulin G1 Fc region of trastuzumab (Clynes et al., 2000). Trastuzumab may internalize the receptor, thereby preventing plasma membrane signaling (Sliwkowski et al., 1999). There is also evidence that trastuzumab decreases cleavage of $p185^{erbB2}$ ectodomain, preventing the formation of a truncated yet highly active receptor remnant (Molina et al., 2001). There have been few attempts to document the relative contributions of these mechanisms in human studies, because study designs demand solutions to the technically challenging problems of accurately monitoring trastuzumab-dependent immune effects, receptor modifications, and changes in the cellular location of ErbB2.

Work with small-molecule ErbB2 tyrosine kinase inhibitors are not complicated by concerns over the contributions of the immune system or complex antibody-mediated receptor effects. Therefore, translational studies have focused on the ability of these alternative ErbB2 targeting agents to disrupt signal transduction. Interestingly, posttreatment biopsies from patients treated with the ErbB2 inhibitor GW572016 often show successful inhibi-

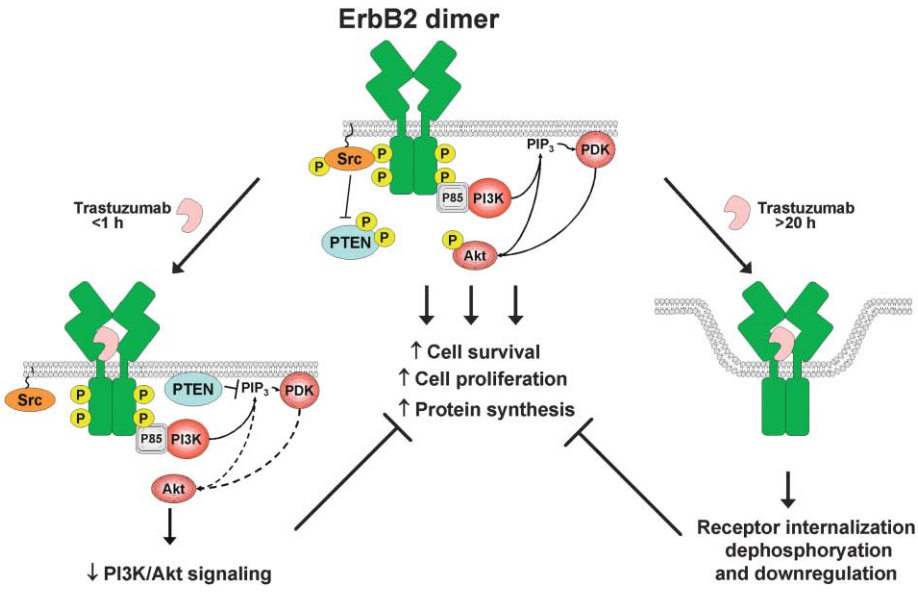

Figure 1. Inhibition of ErbB2/PI3K signaling by trastuzumab

Top: overexpression of ErbB2 results in the formation of homodimers with autophosphorylation of tyrosine residues within the intracellular domain. These phosphorylated tyrosine residues of ErbB2 serve to recruit intracellular signaling molecules, including the non-receptor tyrosine kinase Src and components of phosphatidylinositol 3-kinase (PI3K catalytic subunit and p85 adaptor), to the intracellular domain of ErbB2. Activated PI3K generates 3-phosphorylated phosphoinositide products (PIP3) that directly or indirectly (solid arrows) (through the phosphoinoside-dependent protein kinases [PDK]) lead to the activation of downstream targets of PI3K, including the serine-threonine protein kinase Akt. Upon phosphorylation, Src becomes activated and phosphorylates the tumor suppressor PTEN on tyrosine residues within the PTEN C2 domain, preventing PTEN from translocating to the plasma membrane and dephosphorylating PI3K-generated PIP3. These signaling mechanisms lead to the sustained activation of the PI3K signaling pathway and promote PI3K-regulated cellular processes, including cell proliferation, survival, and protein synthesis. Left top arrow and lower left figure: treatment with trastuzumab results in a rapid downregulation of PI3K signaling. Within minutes of trastuzumab treatment, Src becomes displaced from ErbB2, leading to the dephosphoryation and inactivation of Src. PTEN, no longer held in an inactive state by Src phosphorylation, translocates to the plasma membrane and dephosphorylates PIP3, thereby decreasing the activity of PI3K downstream targets (dashed arrows), including Akt, which is inactivated following dephosphorylation. Top right arrow and lower right figure: in an alternative model, long-term trastuzumab treatment (>20 hr) may result in the internalization of ErbB2, thereby removing ErbB2 from the plasma membrane and preventing its signaling functions. ErbB2 becomes dephosphorylated and degraded upon internalization. Both short- and long-term processes mediated by trastuzumab inhibit cellular functions promoted by activated ErbB2. The relative contribution of these two potential inhibitory effects to the efficacy of trastuzumab remains unclear, but both would be attenuated by the presence of constitutive activation of the PI3K pathway.

tion of receptor autophosphorylation in the absence of the anticipated fall in tumor cell cycle markers and increase in apoptosis (Burris et al., 2003). This suggests that key growth and survival pathways remain active despite successful ErbB2 inhibition. A number of investigators have recently implicated the phosphatidylinositol 3′-kinase (PI3K) pathway in small-molecule ErbB1 inhibitor resistance (Bianco et al., 2003; She et al., 2003). Constitutive PI3K signaling from loss of expression from the PTEN tumor suppressor gene (Eng, 2003) or gain-of-function mutations in PIK3CA (Samuels et al., 2004) are common enough in solid tumors to play a major role in modulating the efficacy of ErbB-directed signal transduction therapy.

The study by Nagata et al. in this issue of *Cancer Cell* (Nagata et al., 2004) is intriguing because it suggests that the efficacy of trastuzumab is similarly dependent on the ability to inhibit PI3K signaling. They report that trastuzumab specifically downregulates PI3K signaling though activation of PTEN, a powerful lipid phosphatase. When present in the plasma membrane, PTEN dephosphorylates the lipid products of PI3K and prevents activation of key PI3K targets. PTEN binds to the plasma membrane via the C2 domain that is negatively regulated by Src-dependent tyrosine phosphorylation. Trastuzumab binding rapidly decouples Src from ErbB2 receptor. The consequent loss of Src activity reduces PTEN C2 domain phosphorylation and allows PTEN to translocate to the plasma membrane and inhibit the PI3K pathway (Figure 1). How trastuzumab achieves this remarkable trick was not explored in the paper, but is likely to be a highly specific because very few ErbB targeting antibodies have an antiproliferative effect. Trastuzumab binds to the juxtamembrane region of the ErbB2 ectodomain (Cho et al., 2003), a region believed to be important for dimerization and transmembrane signaling. Binding of trastuzumab to ErbB2 could therefore result in a conformational change in the intracellular domain of ErbB2 that obscures the Src binding site. Alternatively, trastuzumab may induce perturbations in membrane domains, such as lipid rafts, that alter Src binding or Src access to phosphorylated ErbB2. Another area of future research would be an examination of the effect of trastuzumab on the interactions between ErbB2 and the plethora of other second messengers that dock with the receptor in its activated state, as the effect of trastuzumab on Src binding may not be selective.

If activation of PTEN is the key to the therapeutic action of trastuzumab, then cancer cells that have lost PTEN should show resistance to treatment. In this paper, animal modeling supports this postulate, since an antisense-mediated reduction in tumor PTEN inhibited the efficacy of trastuzumab monotherapy. As expected, the antisense effect was reversed with a pharmacological inhibitor of PI3K. This was not only a good control for the antisense experiment, but the result also carries the message that the efficacy of trastuzumab could be enhanced with inhibitors of the PI3K pathway. Finally, the investigators looked to patient material to correlate PTEN expression (by immunohistochemistry techniques in archived tumor specimens) and the effectiveness of combination treatment with paclitaxel and trastuzumab. It would have been more satisfying to conduct these experiments in the context of trastuzumab monotherapy, since despite their attempts to deal with the issue, PTEN status could still influence paclitaxel efficacy complicating the interpretation of their results. In addition, some PTEN expression subgroups had only a few patients, making firm conclusions difficult to draw because of small sample sizes. Moreover, PTEN expression may resemble a continuous biological variable, so retrospectively redefining expression in terms of PTEN "negative" or "positive" is difficult and has to be interpreted cautiously. Nonetheless, it is fair to conclude that the investigators secured enough preliminary data to pursue the hypothesis further. Currently, thousands of patients are undergoing treatment on trastuzumab adjuvant trials. Formalin-fixed tumor samples should be readily available to test whether trastuzumab resistance is associated with loss of PTEN expression. Their data suggest it might also be worthwhile reconsidering trastuzumab-based chemotherapy for other ErbB2-expressing malignancies if the principle reason for failure were inadequate PI3K inhibition that could be overcome with the addition of a PI3K pathway inhibitor.

The overall allure of this paper is the suggestion that the rigorous pursuit of the status of critical signaling molecules in the tumors we target for trastuzumab and ErbB2 small molecule inhibitors should be a key aspect of our clinical approach to ErbB2-positive breast cancer. Adequate technical approaches for high-throughput biomarker analysis of formalin-fixed tissue by quantitative RTPCR, gene sequencing, and tissue microarray are now at hand and should be applied in a rigorous manner. For optimal success, matching individual tumor signaling anomalies with a customized treatment strategy will ultimately provide the most effective way to take advantage of the ever-increasing list of new biological therapies for cancer.

Robert J. Crowder, Donald P. Lombardi, and Matthew J. Ellis*

Department of Medicine
Division of Oncology
Washington University School of Medicine and Siteman Cancer Center
Campus Box 8056
660 South Euclid Avenue
St. Louis, Missouri 63110
*E-mail: mellis@wustl.edu

Selected reading

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