cell context of molecular alterations that play a role in brain tumors and do provide important further insight into the role of *Bmi1* in brain tumorigenesis. The finding that distinct tumor phenotypes that arise from transformation of different cell populations in the brain suggests that knowing the cell of origin may be important for understanding the prognosis of the tumor. It will likely also be important to determine the signaling mechanisms that subsequently become operational in the different cell contexts to devise new therapies.

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PI3 Kinase Activation and Response to Trastuzumab Therapy: What's neu with Herceptin Resistance?

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Trastuzumab is an established therapy for women with breast cancers that overexpress HER2. Despite its proven benefit in treating breast cancer, not all women derive benefit from this monoclonal antibody, and resistant disease can develop. In this issue of *Cancer Cell*, Berns et al. present evidence that activation of the PI3 kinase pathway, either via loss of the tumor suppressor PTEN or through oncogenic stimulation of PIK3CA, can mediate trastuzumab resistance. This study extends important work of others and forms the rationale for future investigations combining inhibitors of the PI3 kinase pathway in conjunction with trastuzumab therapy.

One of the most successful examples of targeted therapies for epithelial cancers has been the demonstration that breast cancers with amplification of the ERBB2/HER2 oncogene are responsive to trastuzumab (Herceptin), a humanized monoclonal antibody directed against the transmembrane HER2 protein. As a result, patients whose breast cancer cells demonstrate overexpression of HER2 protein by immunohistochemistry and/or gene amplification by fluorescence in situ hybridization (FISH) are candidates for this therapy in both the adjuvant and metastatic settings (reviewed in Hudis, 2007). However, as with many cancer therapies, not all women whose tumors overexpress HER2 will respond to trastuzumab. Indeed, only about one-third of women with newly diagnosed advanced breast cancer that overexpresses HER2 demonstrate tumor regression with trastuzumab monotherapy (Vogel et al., 2002). Also, trastuzumab treatment is not without cost in both economic and human terms. Although it is generally well tolerated, rare but significant cardiac toxicity can develop in patients receiving this therapy, especially when it is given in close proximity to anthracycline chemotherapy. One year of trastuzumab therapy, the current standard regimen in early breast cancer, costs approximately $50,000 (USD). Therefore, the identification of predictive biomarkers that can more accurately select responders or nonresponders is vital to improve the therapeutic index of this agent. Also,

understanding the molecular genetic pathways involved with trastuzumab resistance might allow rational design of combination therapies to overcome the resistance phenotype.

The study by Berns et al. in this issue of Cancer Cell is an additional step in this direction (Berns et al., 2007). Using an unbiased RNA interference (RNAi) genetic screen, these investigators identified the PTEN gene as a mediator of trastuzumab resistance in the HER2-overexpressing breast cancer cell line BT474. Reduced or absent expression of PTEN, a known tumor suppressor, is seen in a significant fraction of human breast malignancies. Because PTEN is a negative regulator of the PI3 kinase pathway (Figure 1), one might suspect that activation of this pathway via other means could also lead to a similar trastuzumab-resistant phenotype. Recently, several groups have demonstrated that hot spot missense mutations in the gene encoding the p110α catalytic subunit of PI3 kinase, PIK3CA, are present in 25%–30% of human breast cancers, resulting in activation of the PI3 kinase pathway (reviewed in Karakas et al., 2006). Armed with this knowledge, Berns et al. showed that ectopic expression of either wild-type or oncogenic mutant PIK3CA led to a trastuzumab resistance phenotype. Although mutant PIK3CA has been shown to be oncogenic, overexpression of wild-type PIK3CA is also oncogenic, as amplification of this gene has been demonstrated in other malignancies such as ovarian cancers. These results nicely complement findings from a study by Nagata et al. wherein loss of PTEN expression led to trastuzumab resistance (Nagata et al., 2004). Although Nagata et al. did not examine the effects of oncogenic PIK3CA, they demonstrated that PI3 kinase inhibitors restored trastuzumab sensitivity in PTEN-deficient cells. Thus, Berns et al. have produced strong confirmatory evidence about the role of signaling via the PI3 kinase pathway in trastuzumab resistance.

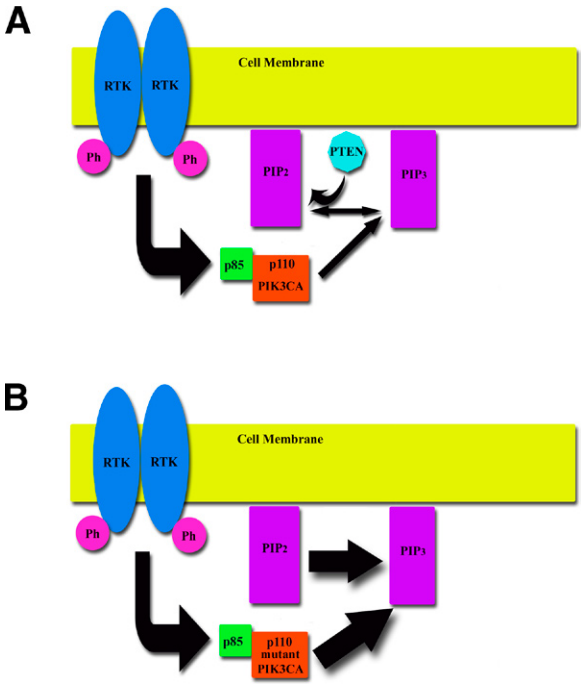

Figure 1. Schematic of PI3 Kinase/AKT Pathway Regulation by PTEN and PIK3CA

(A) Extracellular growth factor receptors (e.g., HER2) are receptor tyrosine kinases (RTK) that can phosphorylate and activate the PI3 kinase/AKT pathway, resulting in a variety of cellular processes. The p110α catalytic subunit of PI3 kinase (PIK3CA) phosphorylates phosphatidylinositol 4, 5 bisphosphate (PIP_2) to phosphatidylinositol 3, 4, 5-triphosphate (PIP_3). This reaction is inhibited by the tumor suppressor PTEN. Ph, phosphorylation.

(B) In the absence of PTEN or the presence of mutant PIK3CA, catalysis is driven from PIP2 to PIP3. This leads to activation of AKT1 and other molecules, resulting in cellular processes that favor neoplastic growth and transformation.

A strength of both the current study and that of Nagata et al. is the attempt to validate preclinical findings in clinical specimens. Berns et al. retrospectively analyzed clinical outcome with respect to PTEN expression and PIK3CA mutations in a convenience sample of 55 primary HER2-overexpressing breast cancers acquired from women who subsequently received trastuzumab-based therapy for advanced disease. Women whose tumors showed low PTEN expression showed a nonsignificant trend toward worse outcome compared with those whose tumors did not. Similarly, breast cancer patients with PIK3CA mutations in their tumors did not fare as well on trastuzumab therapy as those whose tumors had wild-type PIK3CA, but statistical significance was again not achieved. When these two groups were analyzed as one (activated PI3 kinase pathway defined by low PTEN or mutant PIK3CA versus nonactivated PI3 kinase pathway defined by high PTEN and wild-type PIK3CA), those women whose tumors showed an activated PI3 kinase pathway did significantly worse than those whose tumors did not. This finding could argue that it is the activation of this pathway generally rather than alteration in a given gene per se that influences trastuzumab responsiveness. Of note, Nagata et al. did find a statistically significant correlation between low PTEN expression and relative resistance to trastuzumab therapy in another exploratory study. In their study, all 47 HER2-overexpressing patients received trastuzumab with a taxane, whereas the current study included a heterogeneous group of HER2-overexpressing patients receiving trastuzumab with a number of different chemotherapy regimens. Thus, the confounding effects of different chemotherapeutic regimens and/or the small sample size studied could have potentially biased the results of the current study.

How can we interpret the results of Berns et al., and what do they mean for trastuzumab treatment of breast cancer? As with every in vitro and hypothesis-generating pilot study, one cannot apply these results to patient care at this time. However, given the increasing amount of data linking activation of the PI3 kinase pathway with trastuzumab resistance, it would be reasonable to examine the relationship between markers of

PI3 kinase activation and clinical outcome with trastuzumab therapy in a larger group of HER2-overexpressing breast cancer patients. If clinically validated tests can be identified, such analyses might be carried out in samples collected from women with HER2-overexpressing breast cancer who participated in the four randomized clinical trials that established the value of trastuzumab in early breast cancer (Hudis, 2007; Romond et al., 2005; Smith et al., 2007). These studies have the virtue of large size, standardized chemotherapy, well-annotated samples, and meticulous clinical follow-up, all crucial factors in the path to identifying a biomarker that will add value in clinical practice. In addition, these results suggest that combination therapies with PI3 kinase pathway inhibitors and trastuzumab may be more efficacious for some breast cancers with HER2 overexpression. Interestingly, a recent study of lapatinib, a small-molecule dual receptor tyrosine kinase inhibitor of the epidermal growth factor receptor and HER2, showed that PTEN loss was not associated with lapitinib resistance (Xia et al., 2007). This may explain the clinical efficacy of lapatinib in trastuzumab-resistant patients and forms part of the rationale for a newly initiated global trial that will examine the impact of trastuzumab, lapatinib, trastuzumab plus lapatinib, or a sequence of trastuzumab followed by lapatinib for women with early-stage HER2-overexpressing breast cancer.

Further, this study strongly suggests that activation of the PI3 kinase pathway by any means may influence trastuzumab resistance. Therefore, it follows that reliable predictive biomarkers of response to trastuzumab therapy may encompass more than PTEN loss or PIK3CA mutation. For this reason Berns et al. also examined breast cancers for the AKT1 activating mutation recently reported by Carpten et al. (2007) but did not identify this mutation in any of their patient samples. However, PI3 kinase signaling ultimately leads to the activation of several downstream effectors, notably the mammalian target of rapamycin, or mTOR. Therefore, one can speculate that mTOR activation as measured by phosphorylation of downstream targets may also serve as useful predictive biomarkers for assessing response to trastuzumab therapy, a readily testable hypothesis.

Lastly, while new biomarkers for trastuzumab response and resistance such as PTEN expression and PIK3CA mutations are under evaluation, it is important to recall that accurate measurement of HER2 is a vital step in the current management of breast cancer patients, as its expression is the most powerful predictor of trastuzumab benefit at the present time. Multiple clinical studies have demonstrated that evaluation of HER2 expression in routine clinical practice is far from perfect, and new guidelines to guide optimal HER2 evaluation have been issued by the American Society of Clinical Oncology and the College of American Pathologists (Wolff et al., 2007). Researchers across the whole of the translational continuum should always recall the imperative for appropriate development, validation, and dissemination of a biomarker if it is to have an impact on human disease (Henry and Hayes, 2006).

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