

Commentary

Treating breast cancer through novel inhibitors of the phosphatidylinositol 3'-kinase pathway

Robert J Crowder and Matthew J Ellis

Department of Medicine, Division of Oncology, Washington University School of Medicine and Siteman Cancer Center, St. Louis, Missouri, USA

Corresponding author: Matthew J Ellis, mellis@wustl.edu

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Abstract

Recent studies indicate that constitutive signaling through the phosphatidylinositol 3'-kinase (PI3K) pathway is a cause of treatment resistance in breast cancer patients. This implies that patients with tumors that exhibit aberrant PI3K signaling may benefit from targeted pathway inhibitors. The first agents to make it to the clinic are the rapamycin analogs. These compounds inhibit the downstream PI3K effector mTOR (mammalian target of rapamycin). A study presented in this issue of *Breast Cancer Research* suggests that recently developed inhibitors of phosphoinositide-dependent protein kinase 1, a more proximal target of the PI3K pathway, may provide an alternative route to effective PI3K pathway inhibition for breast cancer treatment.

Introduction

The phosphatidylinositol 3'-kinase (PI3K) signaling cascade is involved in regulating many cellular processes that are required for tumorigenesis, including protein synthesis and glucose metabolism, cell survival, proliferation, cell migration, and angiogenesis. Recent investigations indicate that constitutive activation of the PI3K pathway promotes resistance to estrogen receptor and human epidermal growth factor receptor (HER)2 directed therapy for breast cancer patients [1,2]. The major mechanism for abnormal PI3K activation in cancer is thought to be somatic mutation in the genes that encode positive and negative effectors of this pathway. These insights suggest that many breast cancers exhibit a 'genetic dependency' on PI3K pathway mutations that can be exploited for therapeutic gain. For example, abnormal PI3K activation in breast cancer can occur through amplification of the *HER2* gene, the gene product of which is effectively targeted with the monoclonal antibody trastuzumab [3]. In addition, loss of expression of PTEN, a powerful negative regulator of PI3K signaling, or functional loss of PTEN due to *PTEN* gene mutations occurs in up to

50% of breast tumors and results in constitutive PI3K pathway signaling [4,5]. Loss of PTEN expression produces resistance to breast cancer endocrine therapy and trastuzumab treatment, and is a predictor of poor prognosis [1,2,5]. More recently, activating mutations in the *PIK3CA* gene, which encodes the PI3K p110 α catalytic subunit, were found to occur in about 20–40% of breast tumors [6-9]. Interestingly, one study involving a large cohort of breast tumor samples [9] reported that *PIK3CA* gene mutations are mutually exclusive with *PTEN* gene loss, and that *PIK3CA* mutations correlate with HER2 expression and estrogen receptor positive status in breast tumors, although these correlations were not seen in other studies involving smaller samples sizes [7,8]. The *RPS6KB1* gene, which encodes the mammalian target of rapamycin (mTOR) effector P70 S6 protein kinase (S6K), is amplified in approximately 10% of breast cancers [10,11]. *RPS6KB1* gene amplification correlates with HER2 overexpression in breast tumors, possibly due to coamplification of *RPS6KB1* with *HER2* (both genes reside on 17q).

Collectively these observations suggest that genomic alterations leading to constitutive activation of the PI3K pathway exist in the majority of breast cancers. However, considerably more molecular epidemiologic work needs to be performed if we are to appreciate the clinical implications of these gene mutations/amplifications and how they relate to each other in large data sets.

Potential therapeutic interventions

Derivatives of rapamycin, a specific inhibitor of the PI3K pathway target mTOR, are currently under clinical trial development for breast cancer treatment. Although these trials have only recently begun, early results from a phase II

HER = human epidermal growth factor receptor; mTOR = mammalian target of rapamycin; P-Akt = phosphorylated Akt; PDK = phosphoinositide-dependent protein kinase; PI3K = phosphatidylinositol 3'-kinase; S6K = P70 S6 protein kinase.

trial with the rapamycin analog CCI-779 in a cohort of patients with advanced disease [12] demonstrated objective responses. Interestingly, patients responding to treatment had tumors with HER2 overexpression and/or loss of PTEN expression. Alternative small molecule inhibitors are also under investigation. For example, when the antitumor effects of celecoxib did not appear to be entirely due to its function as a cyclo-oxygenase-2 inhibitor, this agent was found to interdict the PI3K pathway by inhibiting phosphoinositide-dependent protein kinase (PDK)-1 [13]. PDK-1 is a direct target of PI3K activity that regulates the activity of downstream kinases, including Akt, mTOR and S6K. Because PDK-1 is a more proximal mediator of PI3K signals than is mTOR, PDK-1 inhibitors target a larger portion of the PI3K pathway and potentially offer a more effective treatment. However, celecoxib is a weak PDK-1 inhibitor, and effective inhibition in patients would require concentrations that are difficult to achieve *in vivo*. Recently, two celecoxib derivatives, namely OSU-03012 and OSU-03013, were shown to be potent PDK-1 inhibitors *in vitro* [14], and the properties of these agents are explored by Kucab and coworkers in this issue of *Breast Cancer Research* [15].

PDK-1 inhibitors attenuate signaling, cell proliferation and cell survival in breast cancer cells

Kucab and coworkers [15] tested the ability of OSU-03012 and OSU-03013 to attenuate PDK-1 dependent signal transduction and cell proliferation/survival in HER2 overexpressing (although not *HER2* gene amplified) breast cancer cell lines. They reported that both analogs effectively downregulated PI3K/PDK-1 dependent signaling at low micromolar concentrations, consistent with the effect of the drugs as selective PDK-1 inhibitors. Both compounds inhibited the phosphorylation of Akt and one of its substrates, namely glycogen synthase kinase-3, as well as the mTOR substrate 4E binding protein 1. These findings confirm that these analogs have the ability to inhibit a wider range of PI3K pathway components than do rapamycin derivatives. Breast cancer cell lines exposed to OSU-03012 and OSU-03013 underwent cell death and exhibited reduced rates of cellular proliferation, although the specific effects with regard to each of these processes were not entirely separable because of the experimental design. The effects of OSU-03012 and OSU-03013 as selective PDK-1 inhibitors must be interpreted cautiously, however, because extensive pharmacologic profiles for these drugs against a panel of signaling enzymes has not been reported. Kucab and coworkers performed experiments suggesting that one of the celecoxib analogues, OSU-03012, exhibits greater specificity for PDK-1, or at least the PI3K arm of the HER2-activated signaling cascade, than does OSU-03013. The former may therefore serve as an attractive lead compound for studies examining the effect of PDK-1 inhibition in preclinical breast cancer modeling studies.

Finally, the authors examined a large cohort of breast tumor samples in an effort to correlate phosphorylated Akt (P-Akt) staining with clinicopathologic variables and HER2 expression. The authors found that P-Akt staining did correlate with higher levels of HER2 expression, but they were unable to correlate P-Akt with overall survival. The failure of P-Akt staining to serve as an adequate prognostic marker in this study is not clear, but this might have resulted from the absence of standardized treatment in the sample cohort. Previous studies reporting P-Akt staining as a poor prognostic marker involved cohorts of patients receiving standardized treatment regimens [16-18], suggesting that the value of P-Akt staining as a prognostic marker may be treatment specific. Moreover, P-Akt staining is a continuous biologic variable, and so quantifying P-Akt staining as 'absent', 'weak', 'moderate', or 'strong' introduces variability into data interpretation. We suspect that there will not be an antibody-based test to provide a reliable shortcut to identify tumors that are responsive to PI3K inhibition, and direct detection of the numerous somatic mutations that drive the PI3K pathway will be necessary.

What is the potential of PDK-1 inhibitors in breast cancer treatment?

Kucab and coworkers [15] mention that preliminary *in vivo* studies indicate that OSU-03012 has significant bioavailability and treatment produces low overt toxicity. In addition, recent preclinical experiments indicated that OSU-03012 treatment may combat imatinib resistance in models of chronic myelogenous leukemia [19] and suggested a use in the treatment of chronic lymphocytic leukemia [20]. OSU-03012 should certainly be pursued in breast cancer treatment. As these studies progress, two important considerations must be taken into account.

First, the determinants of responsiveness of breast cancer cells to PDK-1 inhibitors must be identified. Based on measurements of the sensitivity of breast cancer cell lines to rapamycin analogs, the loss of PTEN expression or the presence of P-Akt or phosphorylated S6K predicts sensitivity to this class of agent [21]. Interestingly, rapamycin-sensitive breast cancer cell lines contain one or more aberrations that are capable of directly activating components of the PI3K pathway, including PTEN loss [21,22], HER2 overexpression, or *PIK3CA* gene mutations [7,9]. Both breast cancer cell lines used by Kucab and coworkers to test the efficacy of PDK-1 inhibitors contain activating *PIK3CA* gene mutations in addition to HER2 overexpression. Therefore, it is unclear whether HER2 overexpression, genetic aberrations within the PI3K pathway, or a combination of these or other factors are predictors of response to PDK-1 inhibitors. These observations underscore the need to identify determinants of breast cancer sensitivity to inhibitors of PDK-1 through the rigorous analysis of multiple markers, which would include PTEN mutation status, HER2 gene amplification, *PIK3CA*

mutational status, and levels of P-Akt and phosphorylated S6K (or gene amplification tests for the relevant genes).

Second, studies designed to test the efficacy of PDK-1 inhibitors either alone or in drug combinations in breast cancer models should be performed in parallel with those using rapamycin derivatives. This will be necessary to compare drug sensitivity profiles, especially in rapamycin-resistant breast cancer models [21], so that the potential application of PDK-1 inhibitors may be defined. Even combinations of the two agents may be justified, depending on the nature of the gene mutation/amplification profile.

Conclusion

Inhibitors of components of the PI3K pathway may provide breast cancer patients with significant therapeutic benefit. Currently, mammalian target of rapamycin inhibitors are being used in clinical trials, and inhibitors to PDK-1 show promise in preclinical development. The central challenge for drug development is to develop predictive biomarkers that accurately identify patients who are likely to benefit from PI3K pathway inhibitors. To do so, it will be imperative to analyze various components of the PI3K pathway and factors that may modify PI3K signaling in future preclinical modeling studies and current clinical trials. The relationships between these markers and cellular response or patient outcome with specific treatment regimens must be codified in order to develop appropriate individualized therapeutic recommendations.

Competing interests

Dr Ellis has received consulting fees from Novartis Pharma regarding the development of the rapamycin analog RAD001.

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