**The Clinical Connection**

**Phosphatidylinositol 3-Kinase in Breast Cancer: Where from Here?**

*Commentary on Barbareschi et al., p. 6064*

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In recent years, the pivotal role that phosphatidylinositol 3-kinase (PI3K) alterations can have in cancer has largely expanded as a result of intensive studies on new functions and alterations in human cancer. We now know that PI3K-Akt pathway is relevant for several fundamental cellular functions, such as cell growth, proliferation, and survival, and, even more important, that PI3K gene alterations are frequent events in many human cancers, including that of colon, breast, brain, liver, stomach, and lung.

PI3Ks are heterodimeric lipid kinases composed of a p110 catalytic and a regulatory p85 subunit encoded by separate genes and alternative splicing. The catalytic PI3KCA subunits are composed of several modular domains: the catalytic lipid kinase domain, the helical domain, a RAS binding domain, and an NH2-terminal domain interacting with the regulatory subunit. Two basic mechanisms that can induce PI3K activity have been described. First, activated cell surface receptor molecules bind the regulatory subunit at SH2 motifs, thus recruiting the catalytic PI3K subunit into specific subcellular localized complexes. A second possibility involves directly the GTPase-Ras binding site, which should bind RAS, but this modality is still a subject of debate (Fig. 1; ref. 1).

Once recruited to the membrane, the p110 catalytic subunit phosphorylates PIP2 to PIP3. The resulting PIP3 serves to recruit phospholipid binding domain containing proteins to the plasma membrane. In particular, Akt is recruited to the membrane and successively fully activated by PDK1 before moving to the cytoplasm and to the nucleus where it phosphorylates a plethora of downstream targets. Survival functions through transcription factors nuclear factor-κB or through phosphorylation of Bad are among the classic downstream effects of activated PI3K-Akt pathway (2); moreover, a game in activation of motility and invasion of human breast cancer cells has been hypothesized (3).

The activity of PI3K is opposed by the action of lipid phosphatases, such as PTEN (phosphatase and tensin homologue deleted in chromosome 10), which removes the 3'-phosphate of PIP3, regenerating PIP2 and attenuating the hypothesis that these two characteristics are mutually exclusive. Conversely, a significant and direct association has been reported between PI3KCA and phosphorylated Akt, target of PI3KCA mutations activating Akt function through its higher phosphorylation (2).

More recently, an increasing body of evidence suggests that a constitutive activation of the PI3K pathway can occur by mutations in the p110α catalytic subunit. Several alterations of PI3KCA gene, a 34-kb gene located on chromosome 3q26.3, with 21 exons coding for a 124-kDa protein, have been described in many human tumors. In particular, multiple studies have shown that these mutations are observed in 18% to 40% of breast cancer (6). A great majority of somatic mutations in breast cancer are missense mutations clustering in exons 9 (E545K) and 20 (H1047R), which encode a part of helical and kinase domains, respectively. These alterations are associated with an increased constitutive kinase activity, thus suggesting that PI3KCA mutations can activate this pathway actually.

Very recently and based on these latter evidence, some authors analyzed the relevance that a mutated PI3KCA status can have on clinical outcome of breast cancer patients. Li et al. (7) screened 250 primary breast cancers, reporting a frequency of 35% of PI3KCA mutations in the C2, helical, and kinase domains; they concluded that the presence of any PI3KCA mutation is an independent factor for worse survival. Maruyama et al. (8) considered 188 primary breast cancers of Japanese women, finding PI3KCA mutations in 29% of cases, 85% of which clustering in exons 9 and 20. Also in this experience, a multivariate analysis confirmed that PI3KCA mutation status was a significant prognostic factor independent from other conventional prognostic factors but, interestingly, predictive of a better prognosis (8). Finally, Barbareschi et al. (9) in the present issue conducted an analysis on 163 consecutive breast cancer patients, finding 28% of mutated cases; however, this last report suggests that mutations located in different exons could have different prognostic value with exon 9 mutations independently associated with a worse prognosis, whereas those in exon 20 with optimal prognosis.

The first comment potentially justifying the controversial results reported by those authors (7–9) concerns the different characteristics of the series of patients they analyzed. In fact, different percentages of node-positive and node-negative patients and different adjuvant chemotherapies and/or hormone therapies administered to about all patients have been included in the three studies. As a consequence, if a predictive value (rather than prognostic) is there for PI3KCA mutations, this could be related to the relevance that PI3K has been reported can have in determining tumor cell hormone (10) and/or chemosensitivity (11) rather than in modulation of tumor aggressiveness. The only further prognostic information

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already available concerns PI3KCA expression in a series of 125 node-negative breast cancer patients who received adjuvant cyclophosphamide/methotrexate/5-fluorouracil reporting no clinical relevance for PI3KCA expression (12), but this work does not evaluate gene functionality.

A further comment refers to the different pathogenetic and functional meaning of different PI3KCA mutations. In vitro experiments suggest that mutations in exons 9 and 20 are the only ones reported as oncogenic, 1 but no other mutations have been tested in gene deletion experiments using somatic knockouts; in particular, the H1047R mutant in exon 20 showed to be the most carcinogenic causing tumors with higher frequency and more aggressiveness (2). However, an increased intrinsic protein kinase activity of PI3KCA has been proven for hotspot mutant either in exon 9 or in exon 20 (13). Barbarechi et al. (9) suggest that mutants in helical and kinase domains could have a profound different effects in phosphorylating the p85α subunit, action that is likely to represent a feedback negative mechanism to shut off a constitutive PI3K activation.

The final comment on potential clinical relevance of PI3KCA concerns the complex pathway in which this kinase is inserted. For most of the upstream and downstream effectors of PI3KCA-Akt pathway (i.e., epidermal growth factor receptor, ErbB2, Akt, 4-EBP1, and P70S6K; refs. 14–18), studies already suggesting a specific prognostic role are available, thus generating the question of what is the clinical relevance that each of these effectors should have when altered within the same cell function pathway. We are completely convinced that variables belonging to similar functional pathways should be checked simultaneously in large series of patients and analyzed as a whole pathway functionality within a decision tree model to permit to understand the hierarchy and the effective clinical relevance that each step alteration can play.

The frequent uncontrolled activation of PI3K-Akt pathway, either due to activation upstream and downstream or gene alterations of PI3K, has made inhibition of PI3K an attractive strategy for therapeutic approaches. The first-generation compounds acting on PI3K have been represented by a series of drugs inhibiting the majority of known PI3Ks and other members of PI3K superfamily without any degree of selectivity for individual PI3K isoforms (19). Among them, wortmannin, quercetin, and LY294002 are PI3K inhibitors at the ATP binding sites. Wortmannin is a fungal metabolite irreversibly acting at nanomolar concentrations; quercetin has also shown a good clinical tolerability in an old phase I study (20) and LY294002 is a flavonoid derivative, competitive, and reversible inhibitor of PI3K ~500-fold less active than wortmannin. However, the selectivity of action of these drugs on PI3K has been recently criticized, being shown that they bind other target than PI3K-related kinases also (21).

Afterwards, more selective compounds acting on p110 catalytic isoform have been synthesized as lead compounds for future development of PI3K isoform-selective inhibitors. These are imidazopyrimidine derivatives against p110α and quinolone and pyridopyrimidine compounds (closely related to LY294002) against α and β isoforms, IC87114, and methyloxanthines active on δ isoforms (22).

Only, few new molecules inhibiting PI3K reached the preclinical study. Among these, the orally available small-molecule KL147 (Exelixis, Inc.), already applied some weeks ago to Food and Drug Administration as a new orally available small-molecule inhibitor of PI3K, is in preclinical studies.

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XL147 slowed tumor growth or caused tumor shrinkage in several preclinical cancer models. Interestingly, XL147 has also been shown to enhance the antitumor effects of several chemotherapeutic agents. An investigational new drug application was filed in March 2007 and a phase I clinical trial is expected to initiate in 2007. Similar in vitro characteristics belong to PX866, an orally and i.v. semisynthetic vyridin analogue (ProliX, Inc.), in preclinical development with investigational new drug application filed in 2006.

Recently, a new clinically viable PI3K inhibitor, SF1126 (Semaphore, Inc.), which is a covalent conjugate of LY294002 containing a peptide-based targeting group, has been synthesized. SF1126 is a highly water-soluble solid compound, helping its administration via i.v. or s.c. injections. In vitro, it converts spontaneously at physiologic pH to LY294002 viable version, and as a produg, it is able to block PI3K without getting unwanted effects on normal cells. The company has announced the recent activation of phase I clinical trial that is conducting under the supervision of D. Von Hoff (at TGen Clinical Research Services at Scottsdale Healthcare’s Virginia G. Piper Cancer Center). The open-label ascending dose trial will assess pharmacokinetics and pharmacodynamics in patients with solid tumors, including endometrial, renal, breast, ovarian, and hormone-refractory prostate cancers, and thought to be driven by PI3K activation or loss of the associated PTEN function. A twin trial is expected to start in the near future for patients with multiple myeloma.

What can we expect from these clinical studies? The premises are extremely relevant for the strategy of PI3K inhibition. As a preliminary consideration, we think that the article of Barbaresci et al. (9) could lead to the hypothesis of the synthesis of new PI3K inhibitors selectively targeting mutants with different biological and clinical functions. In addition, we now know enough to look at this family of drugs not only as potential effective antineoplastic agents. In fact, a further intriguing hypothesis is represented by PI3K inhibitors used as sensitizing agents for apoptotic drugs. Liang showed that in vitro knockdown of PI3K is effective in sensitizing breast cancer cells to gemcitabine- and Taxol-induced apoptosis (23). PI3K inhibitors could also synergize with either doxorubicin or Taxol, resulting in an optimal antineoplastic effect (24). Finally, they could be used for a simultaneous targeting of different cell pathways (25) or for a concorrent inhibition of different steps of the same pathways, so cooperating to more effective inhibition of crucial cell functions.

A more comprehensive knowledge of the signaling intricacies is needed to use the crucial step of PI3K tumor cell activation optimally in the clinical setting.

References
Correction: Article on Phosphatidylinositol 3-Kinase in Breast Cancer

In the article on phosphatidylinositol 3-kinase in breast cancer in the October 15, 2007 issue of *Clinical Cancer Research*, the names of the authors, Angelo Paradiso, Anita Mangia, Amalia Azzariti, and Stefania Tommasi, were listed incorrectly.

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