IN THE SPOTLIGHT

New Routes to Old Places: *PIK3R1* and *PIK3R2* Join *PIK3CA* and *PTEN* as Endometrial Cancer Genes

Sandra Herrero-Gonzalez and Antonio Di Cristofano

Summary: Cheung and colleagues identify *PIK3R1* and *PIK3R2*, the genes encoding the α and β isoforms of the phosphatidylinositol 3-kinase (PI3K) p85 regulatory subunit, as additional mutation targets in endometrial cancer, and describe a novel mechanism leading to PTEN loss. *Cancer Discovery; 1(2);* 106–7. *©2011 AACR.*

Commentary on Cheung et al., p. 170(8).

The phosphatidylinositol 3-kinase (PI3K) enzyme is an obligate heterodimer composed of a regulatory subunit (p85) and a catalytic subunit (p110) (1). The inter-SH2 domain of p85 binds to both the adapter-binding domain and the C2 domain of p110, causing its stabilization and catalytic inhibition, respectively (2). Once recruited to the membrane by the interaction of p85 with a variety of receptors, p110 is activated through a conformational switch and produces phosphatidylinositol 3,4,5-trisphosphate (PIP3), which functions as a cellular second messenger. PIP3 recruits kinases containing a pleckstrin homology domain to the cell membrane, where they are activated. These kinases, the most important of which is AKT, control a multitude of pathways, including cell growth, survival, and metabolism (3). The tumor suppressor lipid phosphatase PTEN hydrolyzes PIP3 to PIP2, thus acting as a functional antagonist of PI3K (4).

Given the range of biological processes controlled by PI3K, it is not surprising that mutations that lead to aberrant activation of the PI3K cascade are frequent events in human cancers (5). In particular, type I, estrogen-related, endometrial cancer (EC) appears to harbor mutations in PI3K pathway members with a particularly high prevalence (6). Previous reports had established high mutation rates for both *PTEN* and the gene encoding the α isoform of p110, *PIK3CA* (7). In this issue of *Cancer Discovery*, Cheung and colleagues (8) present an extremely comprehensive analysis of more than 200 primary ECs that validates *PTEN* and *PIK3CA* as primary mutation targets in this tumor type. Interestingly, the data obtained in this study show that, contrary to what had been previously reported in smaller studies, *KRAS* mutations frequently (>10%) coexist with PI3K pathway mutations, suggesting that simultaneous activation of KRAS and PI3K cooperates to accelerate the tumorigenic process, similar to what we have recently shown in a thyroid cancer model (9). This notion, supported by an initial analysis of the correlation between EC genetic alterations and cell line response to mTOR, PI3K, and mitogen-activated protein kinase inhibitors, will now open the way to a more rational combination of targeted therapies in advanced EC patients.

Most importantly, this study also reveals a relatively high rate of mutations in the genes encoding the α and β isoforms of p85, *PIK3R1* (20%) and *PIK3R2* (5%). The mutation frequency of *PIK3R1* in EC is much higher than previously found in other tumors, and confirms the results of a recent similar analysis performed on a smaller dataset (7). The real novelty is the finding that *PIK3R2* is also frequently mutated in EC, because the mutation rate so far reported in any tumor type was negligible.

Most EC *PTEN* mutations are in heterozygosity, and about half of the $PTEN^{+/-}$ tumors show complete loss of protein expression, supporting the notion that epigenetic and posttranslational mechanisms contribute to PTEN loss during neoplastic transformation. Furthermore, *PTEN* heterozygous mutations frequently coexist with PIK3CA, *PIK3R1*, and *PIK3R2* mutations, and in this case a lower percentage of tumors shows complete loss of PTEN protein, strongly suggesting that activation of PI3K is necessary and sufficient to overcome the activity of the remaining *PTEN* allele.

These compelling genetic data seem to seriously undermine the notion of *PTEN* haploinsufficiency by providing evidence of mechanisms that bypass the need for total PTEN loss to activate PI3K downstream signaling.

A second key finding in the article by Cheung and colleagues (8) comes from the analysis of the effect exerted by PIK3R1 and PIK3R2 mutations on PI3K signaling. Based on the current knowledge of p85's role in controlling p110 activation, it is not unexpected that most *PIK3R1* and *PIK3R2* mutations are gain-of-function and work by removing the inhibitory control of p85 over p110; however, one specific PIK3R1 mutant, E160*, unexpectedly uncovers a different mechanism that leads to constitutive PI3K activation.

It was previously shown that p85α interacts with and increases PTEN activity (10). Cheung and colleagues (8) now show that expression of wild-type p85α, but not p85β, increases PTEN protein levels through stabilization. Expression of the p85α E160* truncation mutant, which cannot bind PTEN, leads instead to reduced levels of PTEN protein due to increased ubiquitination and degradation. It appears that p85α binds

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PTEN as a homodimer, and binding of the mutant p85α to the wild-type protein impairs the ability of the dimer to interact with and protect PTEN.

Although these data, combined with the finding of *PIK3R1* mutations, may contribute one additional mechanism explaining loss of PTEN protein in *PTEN* wild-type or heterozygous tumors, more stringent validation is now necessary using *in vivo* models to convincingly prove that p85α protects PTEN from proteasomal degradation in a physiologically relevant system. For example, it would be interesting to reevaluate the data presented by Luo and colleagues (11) showing that loss of one *Pik3r1* allele increases the number of intestinal polyps but decreases prostate cell proliferation, and has no effect on T-cell hyperproliferation in $Pten^{+/-}$ mice, by comparing the levels of Pten protein in these different tissues showing opposite behavior. Along the same lines, it is important to point out that the levels of Pten protein do not seem to change in the liver of 6-month-old conditional *Pik3r1* mutants (12).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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