# Connecting with an Old Partner in a New Way

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**In this issue of *Cancer Cell*, Hao and colleagues report a non-canonical interaction between the insulin receptor substrate 1 and certain oncogenic variants of the p110α catalytic subunit of phosphoinositide 3-kinase (PI3K). A cell-penetrant peptide that disrupts this interaction downregulates PI3K signaling and inhibits tumor growth in mice.**

More than 5,000 mutations of the p110α catalytic subunit of class IA phosphoinositide 3-kinase (PI3K) in cancers have been reported, placing *PIK3CA* (the gene encoding p110α) among the most commonly mutated oncogenes. So far, all mutations show gain-of-function, leading to increased PI3K activity, signaling, and cell transformation. These mutations are widely distributed throughout p110α, which has sparked the question of whether there are any unifying mechanisms for how these diverse mutations activate PI3K. The activity of the wild-type p110α is normally regulated by the p85 regulatory subunit; a p85 SH2 domain associates with the helical domain of p110α, thereby inhibiting the enzyme's basal activity (Figure 1A). Association of the N-terminal SH2 domain of p85 with tyrosine phosphorylated YXXM motifs in activated receptors or adaptor proteins such as insulin receptor substrate 1 (IRS1) disrupts this inhibitory interaction.

The six isoforms of IRS (IRS1–6) are adaptor proteins that are important for regulating proliferation, metabolism, and differentiation. IRSs are key mediators of insulin receptor and insulin-like growth factor-I receptor functions, and IRSs can be phosphorylated on tyrosine residues by insulin receptor. The canonical role of IRS signaling involves phosphorylated tyrosines (pY) in the C terminus of IRS1 recruiting SH2-domain containing proteins (Taniguchi et al., 2006). Shortly after its discovery more than 20 years ago (Sun et al., 1991), it was shown that tyrosine phosphorylation of YXXM sites in IRS1 results in its interaction with the SH2 domains of the p85 regulatory subunit of PI3Ks, accompanied by PI3K activation. The consequence of p85 SH2 domains binding to pY in IRS1 is disinhibition of p110α. IRS1 is required for the transforming potential of many oncogenes, and several studies have shown that IRS1 overexpression can result in transformation (Dearth et al., 2007). IRS1 can also form many non-canonical interactions that do not involve binding to SH2 domains (Dearth et al., 2007).

One subset of p110α mutations, typified by the hot spot E545K mutation in the helical domain, increases PI3K activity by circumventing the inhibitory influence of the p85 regulatory subunit. The p110α-E545K mutant cannot form the inhibitory interaction with p85 (Miled et al., 2007), so this mutant's activity in vitro resembles the activated wild-type enzyme. In this issue of *Cancer Cell*, Hao et al. (2013) asked whether this disinhibition of p110α-E545K fully explains its phenotype or whether there might be extrinsic contributing factors. They began by carrying out a proteomic analysis to see if there might be partners of p110α-E545K that are unique to this mutant as compared with the wild-type enzyme. They show that p110α-E545K and several other oncogenic p110α mutants form non-canonical interactions with IRS1 that are independent of the p85 regulatory subunit (Figures 1B and 1C) (Hao et al., 2013). This unique p110α-E545K/IRS1 interaction also may be the reason that a previous report based on targeted mode mass spectrometry showed a greater association of p110α-E545K with IRS1 than the wild-type enzyme (Yang et al., 2011).

The ability of mutations broadly distributed throughout the three-dimensional structure of p110α to bind to IRS1 (Figure 1A) suggests that the non-canonical interaction is not a simple contact with only the helical domain. This is reminiscent of the conundrum of how oncogenic mutations distributed throughout the structure of p110α all activate the enzyme. Structural studies of oncogenic variants of p110α have helped to answer this question. These oncogenic mutations cause allosteric changes mimicking the dynamic events that accompany activation of the wild-type enzyme (Burke et al., 2012). Activation-associated allosteric changes are prominent in four p110α regions: the ABD/RBD linker, the C2/iSH2 interface, the helical domain, and a region near the C terminus of the enzyme. Hao et al. (2013) show that mutants in the first three of these regions lead to increased IRS1 association (Figures 1A and 1B). It may be that it is the activated conformation of p110α that associates with IRS1 so that many mutations could lead to this same type of IRS1 binding. However, Hao et al. (2013) have so far examined the characteristics of only the p110α-E545K mutant in detail. Previous studies suggest that this mutation causes conformational changes in the helical domain, the C2/iSH2 interface, and the ABD/RBD linker. This might explain why Hao et al. (2013) found that the C2 domain mutation N345K and the ABD/RBD linker mutant K111N also led to increased IRS1 association (Figure 1A). It will be interesting to see if the consequences of other p110α mutants binding IRS1 are the same as for p110α-E545K, i.e., increased PI3K signaling, membrane association, and tumor volume (Figure 1C). More work is needed to answer key questions about this non-canonical IRS1 interaction: why is it specific to the mutant enzymes, and what is the extent of the interface of the mutant enzymes with IRS1?

Hao et al. (2013) show that the p110α/IRS1 interaction increases association with membranes in cells and increases p110α stability, but it is not clear if these directly result in the increased PI3K signaling. Although IRS1 and p110α-E545K associate in vitro, this does not lead to increased lipid kinase activity. It may be that the activation of PI3K signaling observed in cells is caused by IRS1 stabilizing the p110α-E545K mutant protein, or possibly activation in cells requires the participation of another IRS1 partner. Hao et al. (2013) show that the p110α kinase domain mutant H1047R does not show increased association with IRS1 in unstimulated cells, suggesting that the p110α-H1047R and p110α-E545K mutants function via different mechanisms. This is consistent with observations in cells and in xenografts showing differential effects of these mutations, with the p110α-E545K expression resulting in a more severe metastatic phenotype (Pang et al., 2009; Zhao and Vogt, 2008). Similarly, structural analysis suggests that the p110α-H1047R mutation cannot elicit the same allosteric changes caused by p110α-E545K. Instead, p110α-H1047R causes conformational changes that are restricted to the C-terminal lobe of the kinase domain (Burke et al., 2012).

Remarkably, Hao et al. (2013) have shown that a peptide corresponding to an 18-residue region from the helical domain of p110α-E545K decreases PI3K signaling in cells. Their peptide design includes a "staple" intended to stabilize the helical conformation present in the full-length enzyme structure. As a bonus, this hydrophobic staple also appears to enable the peptide to enter cells. Importantly, treating p110α-E545K-driven tumors in mice with this peptide reduces tumor growth, showing that it may be possible to design therapeutic agents that would specifically inhibit the p110α-E545K oncogene. The observation that several distinct oncogenic mutants show increased association with IRS1 suggests that the IRS1 is targeting a wider interface than just the p110α-helical domain. Allosteric changes produced by these p110α mutations may expose a secondary epitope. Nevertheless, the success of the mutant, stapled helix in blocking this interaction might imply that more drug-like therapeutic agents might also be effective in selectively inhibiting PI3K signaling by targeting the non-canonical IRS1 interface.

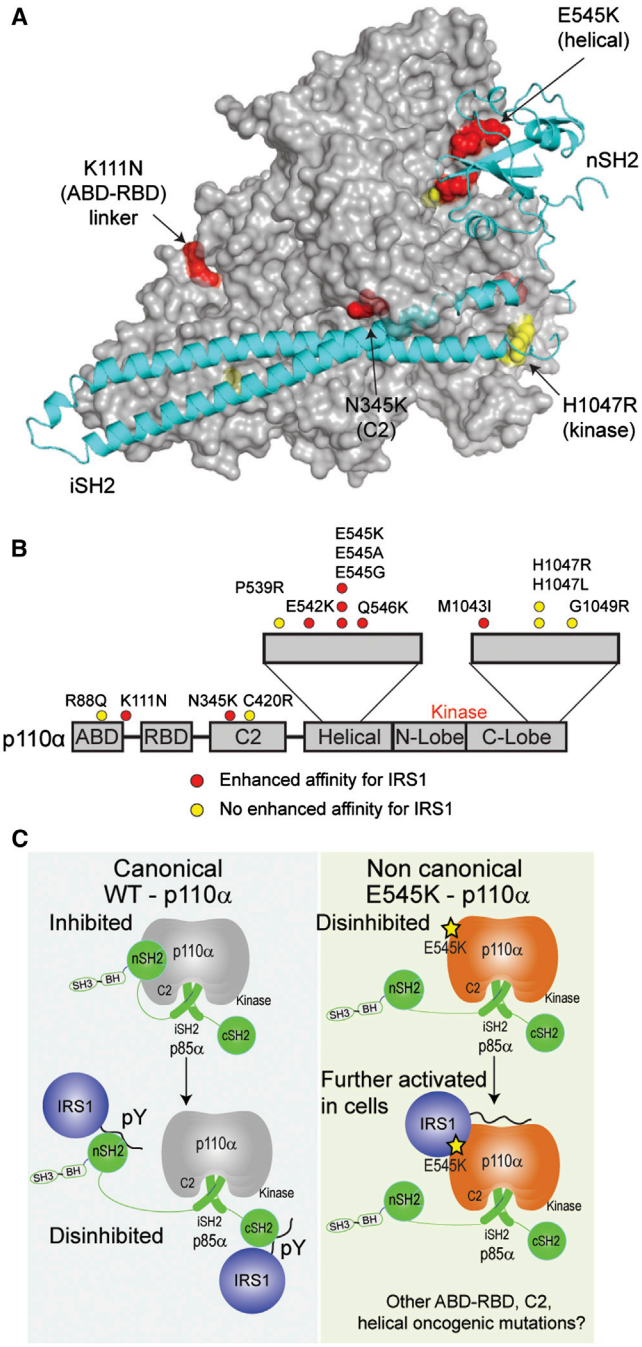


**Figure 1. Non-Canonical Activation of Oncogenic Mutants of PI3Kα by IRS1**

(A) Crystal structure of the nSH2 and iSH2 of p85 (blue) with p110α (gray) (Mandelker et al., 2009), illustrating oncogenic mutants that induced (red) or did not induce (yellow) IRS1 binding.

(B) Domain architecture of p110α and locations of mutants tested by Hao et al. (2013) along with the location of inhibitory contacts between the p110α and p85 subunits.

(C) Non-canonical activation of PI3Kα by IRS1 leads to increased downstream signaling and enhanced tumor volume.

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# A New Mode of RAF Autoregulation: A Further Complication in the Inhibitor Paradox

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**ERK pathway activation in cells expressing wild-type BRAF is a well-reported, clinically-relevant adverse effect of the otherwise impressive response of $BRAF^{V600E}$-mutated melanomas to RAF inhibitors. In this issue of *Cancer Cell*, Holderfield and colleagues show that RAF autoinhibition underpins this paradox, further complicating therapeutic strategies centered around RAF.**

The addiction of cancers to driver oncoproteins has been exploited in the design of novel therapies for cancer treatment; there have been numerous success stories in translating this approach to the clinic. $BRAF^{V600E}$ is a founder, oncogenic driver mutation detected in ~50% of human malignant melanomas. Tumors bearing this mutation are remarkably sensitive to ATP competitive RAF kinase inhibitors, notably vemurafenib (PLX4032) and dabrafenib (GSK2118436) (Flaherty et al., 2010). Resistance to many targeted therapies is associated with longer-term treatment, and, although the initial response to the drug is impressive, certainly in the case of vemurafenib, resistant tumors re-emerge after 2–18 months of treatment.

Apart from acquired drug resistance, the use of RAF inhibitors, unlike other targeted agents, introduces an additional confounding issue that arises due to unique complexities of RAF regulation: ERK pathway activation in cells bearing oncogenic or normally activated RAS. This phenomenon of "paradoxical activation" was initially reported in cancer cell lines bearing *RAS* mutations and was supported by data utilizing a mouse model expressing kinase inactive BRAF (Heidorn et al., 2010). Subsequently, the phenomenon has been shown to account for the emergence of squamous cell carcinomas (SCCs), many of which bear *RAS* mutations, in 15%–30% of vemurafenib-treated human melanoma patients (Su et al., 2012). SCCs are not considered to be a threat to patients as they are well differentiated, nonmetastatic, and relatively easy to remove because they arise on the skin. However, there is always the concern (although not yet proven) that drug treatment may accelerate progression of *RAS*-driven internal lesions that are more difficult to detect. Consequently, there has been a drive to understand the mechanisms underpinning ERK reactivation and to design novel therapies that may circumvent this problem. Paradoxical activation mechanisms have also been proposed to explain drug resistance acquired in $BRAF^{V600E}$ mutant melanomas in situations where a spliced truncated variant of $BRAF^{V600E}$ lacking a RAS-binding domain is expressed (Poulikakos et al., 2011).

Inhibitor-induced ERK pathway activation in cells expressing $BRAF^{WT}$ requires RAF dimerization and transactivation promoted by active RAS (Hatzivassiliou et al., 2010; Heidorn et al., 2010; Poulikakos et al., 2010). Initial reports proposed that inhibitor-bound BRAF acts as a scaffold to enhance CRAF activity at the plasma membrane following RAS-induced BRAF-CRAF heterodimer formation. However, in a separate study, Poulikakos et al. (2010) demonstrated RAF homodimer and heterodimer formation in response to inhibitor and proposed a model whereby inhibitor binding to one protomer of the RAF homo- or heterodimer leads to its inhibition but causes transactivation of the other inhibitor-free protomer. Furthermore, transactivation was not diminished in BRAF null cells, highlighting a dominant role of CRAF homodimers. In $BRAF^{V600E}$ tumors, RAS is inactive and BRAF remains a monomer, transactivation of $RAF^{WT}$ is not possible, and inhibition of $BRAF^{V600E}$ becomes dominant. The importance of dimerization was supported by analysis of dimerization mutants ($CRAF^{R401A}$ and $BRAF^{R509H}$) that prevented inhibitor-induced transactivation (Hatzivassiliou et al., 2010). Overall, these studies led to the hypothesis that development of non-ATP competitive inhibitors that prevent RAF dimerization would prevent the adverse transactivation effects.

A novel mechanism involving RAF autoregulation has now been identified by Holderfield et al. (2013), in this issue of *Cancer Cell*, which changes the therapeutic approach significantly. This group developed nine compounds with varying