PI3K Regulatory Subunits Lose Control in Cancer

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The PI3K signaling axis is frequently activated in cancer resulting from inactivation of its negative regulator PTEN or activating mutations of the p110α catalytic subunit of PI3K. In this issue of *Cancer Cell,* Jaiswal et al. report that mutations in the p85α regulatory subunit of PI3K can also activate this pathway in cancer.

Phosphoinositide 3-kinase (PI3K) signaling is often constitutively switched on in cancer, providing tumor cells with enhanced capacities for growth, proliferation, survival, and migration. PI3Ks generate lipid second messengers inside the cell to channel signals from upstream activators at the plasma membrane, such as receptor tyrosine kinases and Ras, to downstream effectors, such as the Akt protein kinase and its many targets. Clinical trials in cancer with small-molecule inhibitors against PI3K are now in progress.

In 2004, DNA sequencing of the kinase domains of the eight PI3Ks and eight PI3K-related genes in a large set of mainly colon cancer samples revealed the presence of frequent somatic (i.e., cancer cell-specific) mutations only in the *PIK3CA* gene, which encodes the p110α catalytic subunit of the class I PI3Ks (Samuels et al., 2004) (Figure 1). Follow-up studies have confirmed the presence of these *PIK3CA* mutations in many types of solid tumors.

With the exception of the Ras-binding domain, *PIK3CA* mutations in cancer are found throughout the p110α molecule, clustering in so-called "hot-spot" regions in the kinase domain and the adjacent helical domain (Figure 1). The molecular mechanisms by which these mutations activate p110α are just beginning to be unraveled. One mechanism is a possible facilitation of the binding of p110α to membranes in the case of the H1047R mutation (Mandelker et al., 2009). Another mechanism is through modulation of an inhibitory interaction of p110α with its regulatory subunit encoded by the *PIK3R1* gene, as detailed below.

A subset of the p110 catalytic subunits of PI3Ks, namely p110α, p110β, and p110δ (encoded by *PIK3CA*, *PIK3CB*, and *PIK3CD*, respectively), constitutively bind to an SH2 domain-containing protein called p85. There are three genes encoding for five p85 species: *PIK3R1* encodes p85α, p55α,ₛ and p50α; *PIK3R2* encodes p85β; and *PIK3R3* encodes p55γ (Figure 1). p85s stabilize the intrinsically unstable p110 proteins, but also keep them in a low activity state. Through their SH2 domains, the p85s bind phosphotyrosines that are generated in receptors and in receptor-associated proteins upon cellular activation. This recruitment relieves the p85-mediated inhibition of p110 and brings the catalytic subunits in contact with their lipid substrates at the membranes.

One well-defined mechanism of activation of p110α by mutation in cancer is that of the E545K hotspot mutation in the helical domain of p110α (Figure 1). This mutation relieves the basal repression of p85α on p110α through disruption of an inhibitory charge-charge interaction between the two proteins. Thus, p85α no longer inhibits p110α-E545K. Interestingly, through artificial introduction of specific mutations in p85α, it was possible to restore the inhibition of p85α on p110α-E545K (Miled et al., 2007). This suggested that naturally occurring *PIK3R1* mutations should in principle also be capable of activating wild-type p110α.

Over the years, there have been several reports of low-frequency mutations or deletions in *PIK3R1* in cancer, and this has now been confirmed by recent high throughput DNA sequencing efforts (Parsons et al., 2008; TCGA, 2008; Wood et al., 2007) and by Jaiswal et al. (2009), in this issue of *Cancer Cell*. Interestingly, many (but not all) of these mutations cluster in the two SH2 domains and the so-called inter-SH2 domain they flank, through which p85 binds p110 (Figure 1). Jaiswail et al. characterized a number of these p85α mutants in detail, including D560Y, N564D, and QYL579 delL. The latter encodes a p85α mutant that has a two amino acids deletion in the iSH2 domain but maintains p110-binding activity. They found that these mutants retain their ability to stabilize p110α but lose their inhibitory activity. Further biological studies revealed an enhanced capacity of these p85α mutants to increase Akt signaling, cell survival, anchorage-independent cell growth, and tumorigenesis in nude mice.

A remarkable pattern of selectivity of PI3K mutation in cancer is starting to emerge. Compared to p110α and p85α/p55α/p50α, the frequency of mutation in other catalytic-regulatory subunits is extremely low or absent (Jaiswal et al., 2009 and Figure 1). This is somewhat surprising, given that p110β and p85β are also highly expressed in tissues and cancer cells (Geering et al., 2007). Moreover, upon artificial coexpression in cells, each p110 isoform can bind any p85 species, but it is not clear whether endogenous p110 isoforms have a preference for specific p85 isoforms. Taken together, these data indicate that p110α, possibly when in complex with p85α, has cancer-relevant functional characteristics that are not shared by other PI3K complexes.

Interestingly, Jaswail et al. show that *PIK3R1* mutations can also activate p110β and p110δ. Thus, even when not mutated in cancer, p110β and p110δ can be activated by mutation of p85α, clearly broadening the mechanisms by which the PI3K pathway can be activated in cancer. p110β and p110δ can be overexpressed in human cancer and can induce characteristics of transformation in chicken embryo fibroblasts (Kang et al., 2006). If co-occurring in the context of enhanced p110β or p110δ expression, mutated p85α could even further enhance the activities of these nonmutated catalytic subunits. Of interest from a therapeutic perspective, all mutant p85α/p110
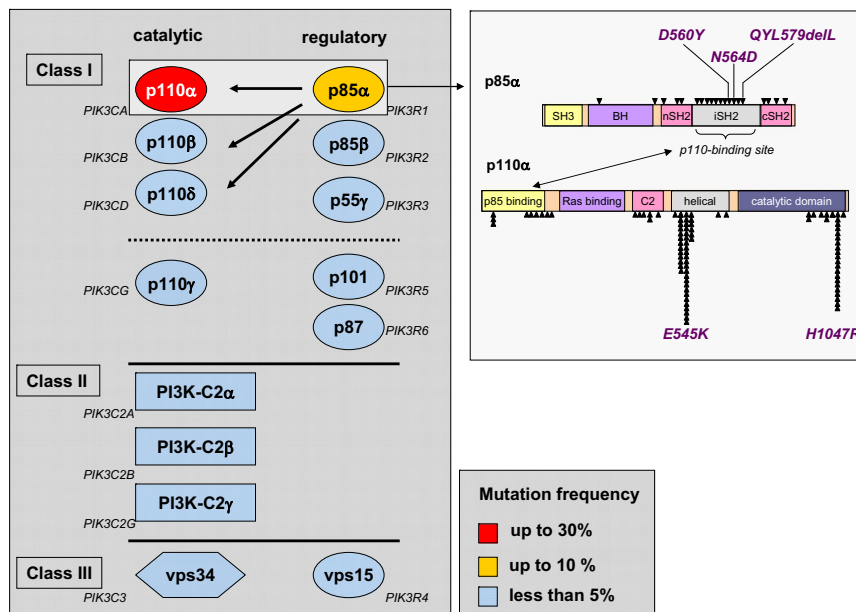
Figure 1. Cancer Mutations in PI3K Family Members
The left panel shows the frequency of mutation in cancer in the different PI3K family members. The right panel shows the location of mutations (indicated by arrowheads) in p110α and p85α. The mutations that are discussed in the text are spelled out. Not shown are the p55α and p50α proteins, which are encoded by transcripts from the *PIK3R1* gene through alternative promoter usage. Compared to p85α, p55α and p50α contain the same SH2 domains and the iSH2 domain but a smaller N-terminal extension.

complexes tested were found to retain their sensitivity to PI3K inhibitors that were generated against the wild-type p110 proteins. In other words, mutation of p85α would not necessarily lead to therapeutic resistance.

An important question raised by the work of Jaiswal et al. is whether these "oncogenic" mutations in p85α are driver mutations in cancer or secondary mutations that help tumor development once cells have acquired a tumor-prone genetic profile by other means. How they are implicated in cancer may also depend on the tissue and cell type, as suggested by the pattern of co-occurrence with other oncogenic alterations in the PI3K pathway. For example, in colon cancer, most *PIK3CA* mutations do not co-occur with *PIK3R1* mutations. However, when a *PIK3R1* mutation is found, half of these also have a *PIK3CA* mutation. Moreover, 22% of the samples with a *PIK3R1* muta-

tion also have a *PTEN* mutation (Jaiswal et al., 2009). To the contrary, in glioblastoma, *PIK3R1* mutations do not co-occur with *PIK3CA* mutations (Parsons et al., 2008; TCGA, 2008) and very rarely with *PTEN* mutations, despite the high frequency of the latter in this tumor type. However, these data need to be interpreted carefully because finding two mutations in the same tissue sample does not necessarily mean that they have occurred in the same cancer cell.

Can the presence of p85α mutations be used as a diagnostic marker to identify cancers that are dependent on PI3K, and thus identify good candidates for treatment with PI3K pathway inhibitors? Ongoing studies suggest that there is no good correlation between the presence of p110α mutations and sensitivity to PI3K inhibitors, at least in cultured cancer cell lines. The same applies for inactivation of PTEN. It is therefore not entirely

clear what the predictive value of the p85α mutations will be, in terms of inhibitor sensitivity, and it is quite likely that other lesions may have a modulating effect on pathway sensitivity. The real challenge will be to build a comprehensive genetic landscape surrounding PI3K alterations in different cancers in order to better predict sensitivity to inhibitors targeting this pathway. Such efforts are in progress (Sos et al., 2009).

REFERENCES

Geering, B., Cutillas, P.R., Nock, G., Gharbi, S.I., and Vanhaesebroeck, B. (2007). Proc. Natl. Acad. Sci. USA *104*, 7809–7814.

Jaiswal, B.S., Janakiraman, V., Kljavin, N.M., Chaudhuri, S., Stern, H.M., Wang, W., Kan, Z., Dbouk, H.A., Peters, B.A., Waring, P., et al. (2009). Cancer Cell *16*, this issue, 463–474.

Kang, S., Denley, A., Vanhaesebroeck, B., and Vogt, P.K. (2006). Proc. Natl. Acad. Sci. USA *103*, 1289–1294.

Mandelker, D., Gabelli, S.B., Schmidt-Kittler, O., Zhu, J., Cheong, I., Huang, C.H., Kinzler, K.W., Vogelstein, B., and Amzel, L.M. (2009). Proc. Natl. Acad. Sci. USA *106*, 16996–17001.

Miled, N., Yan, Y., Hon, W.C., Perisic, O., Zvelebil, M., Inbar, Y., Schneidman-Duhovny, D., Wolfson, H.J., Backer, J.M., and Williams, R.L. (2007). Science *317*, 239–242.

Parsons, D.W., Jones, S., Zhang, X., Lin, J.C., Leary, R.J., Angenendt, P., Mankoo, P., Carter, H., Siu, I.M., Gallia, G.L., et al. (2008). Science *321*, 1807–1812.

Samuels, Y., Wang, Z., Bardelli, A., Silliman, N., Ptak, J., Szabo, S., Yan, H., Gazdar, A., Powell, S.M., Riggins, G.J., et al. (2004). Science *304*, 554.

Sos, M.L., Fischer, S., Ullrich, R., Peifer, M., Heuckmann, J.M., Koker, M., Heynck, S., Stuckrath, I., Weiss, J., Fischer, F., et al. (2009). Proc. Natl. Acad. Sci. USA *106*, 18351–18356.

TCGA. (2008). Nature *455*, 1061–1068.

Wood, L.D., Parsons, D.W., Jones, S., Lin, J., Sjoblom, T., Leary, R.J., Shen, D., Boca, S.M., Barber, T., Ptak, J., et al. (2007). Science *318*, 1108–1113.