

## Targeting therapeutic liabilities engendered by *PIK3R1* mutations for cancer treatment

The regulatory subunit of PI3K, p85 $\alpha$  (encoded by *PIK3R1*), binds, stabilizes and inhibits the PI3K p110 catalytic subunit. Functional characterization of *PIK3R1* mutations has identified not only hypomorphs with reduced inhibition of p110, but also hypomorphs and dominant negative mutants that disrupt a novel regulatory role of p85 $\alpha$  on PTEN or neomorphs that activate unexpected signaling pathways. The diverse phenotypic spectrum of these *PIK3R1* driver mutations underscores the need for different treatment strategies targeting tumors harboring these mutations. This article describes the functional consequences of the spectrum of *PIK3R1* driver mutations and therapeutic liabilities they may engender.

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Tumorigenesis is a multistep process that involves accumulation of genomic aberrations resulting in signaling pathway deregulation. Cancer cells depend on one or more deregulated pathways to initiate and maintain malignant phenotypes. This concept of ‘oncogene addiction’ provides the rationale for targeted therapy blocking signaling pathways essential for maintenance of the malignant phenotype. Targeted cancer therapy, which interferes with the functional consequences of specific molecular abnormalities, has the potential to generate improved antitumor activity and lower systemic toxicity than conventional therapies. The identification of potential therapeutic targets is facilitated by comprehensive genomic profiling of tumors provided with robust functional genomics to characterize the functional consequences of genomic aberrations [1,2].

Aberrations of members along the PI3K pathway are among the most frequent driver events across different cancer lineages. The class I PI3Ks constitute a lipid kinase family that catalyze the production of phosphati-

dylinositol-3,4,5-triphosphate (PIP3) from phosphatidylinositol-4,5-bisphosphate (PIP2) at the cell membrane [3]. PIP3 is a lipid second messenger that recruits PH domain containing molecules including the proto-oncogene AKT for activation. The class IA PI3K is an obligate heterodimer composed of a catalytic subunit (p110) and a regulatory subunit (p85) [4]. The class IA catalytic PI3Ks are comprised of three isoforms (p110 $\alpha$ , p110 $\beta$  and p110 $\delta$ ). p110 $\alpha$  and p110 $\beta$  are ubiquitously expressed whereas p110 $\delta$  is more abundant in hematopoietic cells [5]. There are five regulatory isoforms which are recognized as p85-related regulatory subunits: p85 $\alpha$  (and its splicing variants p55 $\alpha$  and p50 $\alpha$ ), p85 $\beta$  and p55 $\gamma$  [6].

Although the catalytic reaction depends on the activity of p110, p85 plays a critical role in enabling the pathway activation by stabilizing p110 providing a pool of p110 that can be activated by intercellular signaling molecules. p110 molecules not associated with p85 are prone to thermo degradation [7]. The association with p85 also inhibits the activity

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of p110 but the inhibition is relieved upon RTK activation and recruitment of p85 to phosphotyrosine residues in the RTK or intracellular linker molecules [7,8]. Binding of the SH2 domains of p85 to phosphotyrosine relieves the inhibitory effect of p85 on p110. This process results in spatial regulation of PI3K activity. p85 therefore allows ligand-dependent activation of the PI3K pathway, which then contributes to widely divergent physiological processes including cell proliferation, survival, cell cycle progression, differentiation, protein synthesis, motility and metabolism [9–11].

### The PI3K pathway in cancer: aberrations & therapeutic opportunities

In cancer, the PI3K pathway can be aberrantly activated through multiple mechanisms including crosstalk with other signaling pathways or ‘intrinsic’ hyperactivation due to genomic aberrations [12–17]. Common genomic aberrations across cancer types in the PI3K pathway include loss of the negative regulator PTEN via gene mutations, copy number loss or promoter hypermethylation and mutations or gene amplifications of *PIK3CA* (which encodes the p110 $\alpha$  catalytic subunit). Specifically, the *PIK3CA* and *PTEN* genes are the 2nd and 3rd most frequently mutated gene respectively across 12 cancer types sequenced in The Cancer Genome Atlas (TCGA) [18]. *PTEN* is within the 9th most significantly deleted region whereas *PIK3CA* is within the 4th most amplified region across 11 cancer types [19]. There are genomic aberrations of other core members such as mutations in *PIK3R1* (p85 $\alpha$  regulatory subunit) or *PIK3R2* (p85 $\beta$  regulatory subunit) and mutation or amplification of AKT isoforms (*AKT1*, *AKT2* and *AKT3*) [18]. Aberrations are also detected in the upstream inputs (e.g., EGFR, FGFR) or downstream effectors of the pathway, such as MTOR, TSC1, TSC2 and RICTOR.

Being a critical player in tumorigenesis and the most frequently hyperactivated signaling pathway in cancer, pharmacological inhibition of the PI3K pathway is, therefore, considered to be among the most promising strategies for targeted cancer therapy [14,20]. Many inhibitors have been developed targeting components of the PI3K pathway and many of them have been introduced into clinical trials [21,22]. Since the majority of the components in the PI3K pathway are kinases, the pathway therefore presents a group of ‘druggable’ targets using small molecule inhibitors. Direct inhibition of the PI3K or inhibition of downstream kinases through a variety of small molecules with different mechanisms of action have been developed and entered clinical trials. These include small inhibitors targeting all PI3K isoforms, specific PI3K isoforms, dual PI3K/mammalian target of rapamycin (mTOR), mTOR, or

AKT [23,24]. Representative first-generation inhibitors that have demonstrated activities in a range of tumor lineages include GDC-0941 (a pan-PI3K inhibitor) [25], NVP-BEZ235 (a dual PI3K/mTOR inhibitor), Rapalogs (mTOR inhibitors) [26,27] and MK-2206 (AKT inhibitor) [28]. Isoform-specific PI3K inhibitors offer the potential advantage of on-target blockade and minimizing off-target effects due to inhibition of other isoforms as in the case of pan-PI3K inhibitors. Encouragingly, GS-1101 (p110 $\delta$ -specific, previously known as CAL-101) a p110 $\delta$ -specific inhibitor has shown remarkable success in the clinic for the treatment of hematopoietic malignancies that express relatively high levels of p110 $\delta$  [29]. In addition, an array of second generation and more potent agents targeting the PI3K pathway are in development or early clinical trials [30,31]. Blocking upstream signaling from RTKs provide an indirect approach to inhibiting the PI3K pathway. The first-in-class approaches that have been used in the clinic include trastuzumab (a monoclonal antibody that blocks the Erb2/HER2 receptor) [32] and imatinib (small molecule kinase inhibitor that inhibits the BCR-Abl, c-KIT, and PDGF-R tyrosine kinases) [33]. However, although, with the exception of GS-1101, PI3K pathway inhibitors have demonstrated limited activity as single agents in early trials, combinations with hormonal manipulation in breast cancer shows major promise. Thus it is likely that the potential of PI3K pathway inhibition will only be fulfilled by the implementation of rational drug combinations.

In addition to *in vitro* or *in vivo* evidence, clinical studies have suggested that inhibition of the PI3K pathway may be more effective in cancer patients with genomic aberration in the pathway [34]. Molecular aberrations in cancer genes can be utilized as ‘signals’ of potential resistance or sensitivity to specific targeted therapies. Further, the specific mutation present in a cancer gene can have different functional consequences as well as determine therapeutic sensitivity. For example, cancer patients with *PIK3CA* H1047R mutation showed better response to inhibitors targeting PI3K, mTOR or AKT than individuals with wild-type (WT) *PIK3CA* or other *PIK3CA* mutations [35]. PTEN aberrations including loss of PTEN protein and/or *PTEN* mutation were also associated with higher efficacy in PI3K pathway targeting [36]. Due to the surge in high-throughput sequencing of cancer genomes and the increased availability of genomic data, incorporation of the genomic information into cancer patient management is increasingly feasible. The key to successful translation into clinical benefit for cancer patients relies on understanding the functions of mutations as well as the therapeutic liabilities that these specific mutations engender.

## **PIK3R1 is one of the cancer genes in the PI3K pathway**

There has been much effort in characterizing the functional consequence of *PIK3R1* mutations recently. The *PIK3R1* gene is the 12th most commonly mutated gene across cancer lineages and several mutation hotspot regions are detected, providing justification for exploring approaches to target the functional consequences of *PIK3R1* mutations in cancer patients. Somatic mutations in *PIK3R1* are particularly prevalent in endometrial cancer (20 and 34% in our and TCGA datasets, respectively [37,38]), glioma (11%; TCGA [39]) and colon cancer (4.5–10%; TCGA [40] and Genentech [41]). One major effort is to distinguish a ‘driver’ mutation that functionally contributes to tumor behavior from biologically neutral ‘passenger’ mutations resulting from instability of cancer genomes. A subset of *PIK3R1* mutations detected are deemed functional and targeting these driver mutations, as compared with passenger mutations, has the potential to benefit cancer patients. In this article, we review the mechanisms by which different p85 $\alpha$  driver mutations exert their oncogenic effects and the biological consequences of these drivers. The *PIK3R1* driver mutations functionally characterized so far can be categorized as hypomorph (loss-of-function mutant), dominant negative that inhibits the activity of WT p85 $\alpha$ , or neomorph (gain-of-novel-function mutant that has completely distinct activity from WT p85 $\alpha$ ). Here we also discuss preclinical data that may shed light on the therapeutic strategies in targeting *PIK3R1* mutated cancers. Although p85 $\alpha$  is not directly druggable, the driver mutations activate downstream signaling pathways and these downstream effectors represent potential therapeutic targets.

## **Functional & therapeutic relevance of PIK3R1 driver aberrations in cancer**

### **Hypomorphic mutants with reduced inhibition of p110**

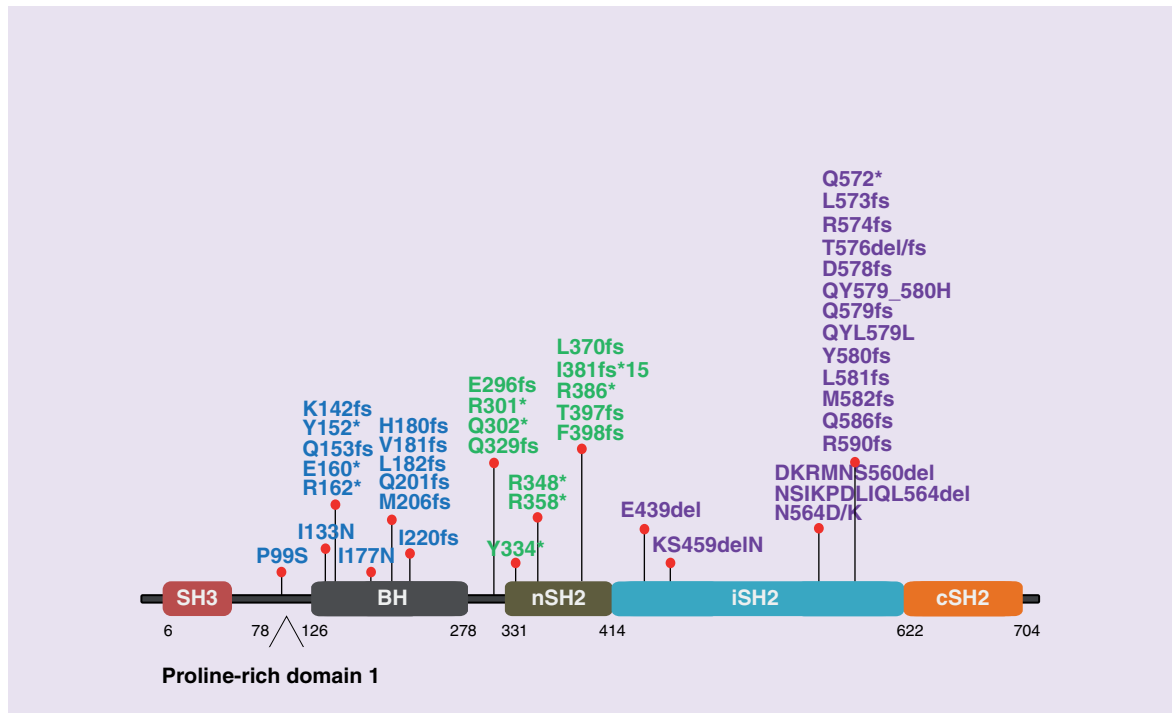
p85 $\alpha$  itself has no kinase activity [42] and its functions are mediated by interaction with protein partners with the most well known binding partner being p110. The nSH2 and iSH2 domains of p85 $\alpha$  (see **Figure 1** for the domain structure of p85 $\alpha$ ) bind the helical and C2 domain of p110, respectively, to inhibit the catalytic activity of the p110 isoforms [43–45]. Two obvious hotspot regions of *PIK3R1* mutations locate within the iSH2 domain (**Figure 1**). These hotspot mutations can be found in multiple cancer lineages, and they represent the first group of *PIK3R1* mutations that were functionally characterized. The underlying mechanism of these *PIK3R1* driver mutations appears to depend on disrupting the iSH2–C2 contact interface, thereby relieving the inhibitory effect on p110 [44,46].

These mutants retain the ability to bind and stabilize p110, leading to hyperactivation of the PI3K pathway in cancer. This subset of p85 $\alpha$  mutants can be referred to as hypomorph.

These p85 $\alpha$  hypomorphic mutations include point mutations (D560Y, N564D), small deletions (E439del, KS459delN, DKRMNS560del, T576del, QYL579L) and frameshift or non-sense mutations (Q572\*, R574fs) resulting in translation of truncation proteins [44,46–48]. No change in the binding activity between the p85 $\alpha$  mutants and p110 $\alpha$  or p110 $\beta$  has been detected. *In vitro* lipid activity assays showed that p110 $\alpha$  coupled to these mutants had higher kinase activity than WT p85 $\alpha$ -bound p110 $\alpha$ . Several postulates based on the crystal structures and computational modeling of the p85 $\alpha$ -p110 $\alpha$  heterodimer have been proposed to explain the loss of the inhibitory effect. The mutants KS459delN and DKRMNS560del most likely disrupt the  $\alpha$ -helical structural conformation of the iSH2 domain that mediates the inhibitory interaction with the C2 domain of p110 [46]. Truncation of p85 $\alpha$  after residue 571 may destabilize a contact between the iSH2 domain and the C2 domain of p110 $\alpha$  [49]. The p85 $\alpha$  amino acid residues D560 and N564 of the iSH2 domain are within the hydrogen bonding distance of N345 of the C2 domain of p110 $\alpha$ . This interaction is required for inhibition of p110 $\alpha$  and the observation that the p110 $\alpha$ -N345 mutant is also oncogenic provides support for this mechanism [44,47]. Consistent with the predicted mechanism of action, the effects of these hypomorphic p85 $\alpha$  mutants were not additive with that of p110 $\alpha$ -N345 [44,50].

The p85 $\alpha$  iSH2 domain mutants lead to constitutive activation of PI3K pathway as demonstrated by increase in phosphorylation of downstream effectors including AKT. The PI3K pathway activation was associated with an increase in transforming activity of multiple cell models expressing these mutants. For example, in glioma models, three p85 $\alpha$  driver mutants (DKRMNS560del, R574fs and T576del) significantly promoted anchorage-independent growth potential in soft agar *in vitro* and tumorigenicity in intracranial xenografts *in vivo* [48]. R574fs and T576del promoted IL3-independent growth of the IL3-dependent Ba/F3 cells [37]. D560Y and N564D induced anchorage-independent growth of NIH 3T3 cells and leukemia-like disease in mice [44,47]. Further, KS459delN, DKRMNS560del, R574fs and T576del increased the transformation potential of chicken embryo fibroblasts [46].

Given the activation of PI3K pathway signaling by p85 $\alpha$  hypomorphic mutants, it may not be surprising that cells harboring these mutants become addicted to the PI3K pathway and are, therefore, sensitive to



**Figure 1.** *PIK3R1* mutations that are functionally verified or potentially functional based on locations. Blue coded mutations potentially involved in p85 $\alpha$  homodimerization and/or PTEN binding. Green coded mutations are neomorphs that activate MAPK signaling pathways. Purple coded mutations disrupt inhibition of p110 by p85 $\alpha$ .

inhibitors targeting the pathway. Ba/F3 cells expressing DKRMNS560del, R574fs, or T576del were more sensitive to an AKT allosteric inhibitor MK-2206 than cells expressing WT p85 $\alpha$  [48]. Another independent study showed that KS459delN or DKRMNS560del rendered chicken embryo fibroblasts sensitive to the mTOR inhibitor rapamycin [46]. Interestingly, these cells were also sensitive to inhibition of p110 $\alpha$  (by the isoform specific inhibitor A66), but not sensitive to inhibition of p110 $\beta$  (TGX-221) or p110 $\delta$  (IC87114) [46]. This suggested p110 $\alpha$  is the major class IA p110 isoform that mediates the phenotypic changes induced by the p85 $\alpha$  mutants at least in this particular cell type. p110 $\delta$  is unlikely involved in the PI3K signaling in this cell type because its expression was undetectable [46]. Although binding between p110 $\beta$  with WT or the mutated p85 $\alpha$  could be detected, the lack of response to p110 $\beta$  inhibition can be due to several potential mechanisms. It is possible that the interaction of p85 $\alpha$  mutants with p110 $\alpha$  and p110 $\beta$  are different and that the effects of p110 $\alpha$  on the PI3K pathway are dominant over p110 $\beta$  when both isoforms are expressed. Moreover, the mechanisms of upstream activation are different between the two isoforms. Unlike p110 $\alpha$ , p110 $\beta$  is activated downstream of both receptor tyrosine kinases and G-protein coupled receptor signaling [51,52]. p110 $\alpha$ /p85 $\alpha$  is more responsive to phosphorylated RTK than p110 $\beta$ /p85 $\alpha$  [45]. Further,

p110 $\beta$  is more tightly regulated than p110 $\alpha$  [45,53]. Apart from the inhibitory contacts mediated by the p85 $\alpha$  nSH2 and iSH2 domains on both p110 $\alpha$  and p110 $\beta$ , a third inhibitory interaction that involves contact between the cSH2 domain of p85 $\alpha$  and the kinase domain of p110 $\beta$  has been suggested [45,53]. Therefore, mutation of amino acid residues at the cSH2 interacting interface may be required to relieve the inhibition of p110 $\beta$ . Along this line, it has been shown that engineered p85 $\alpha$  mutants at the cSH2 domain (Y677A or E675A) relieved cSH2-mediated inhibition of p110 $\beta$  [53]. The kinase domain/cSH2 contact exists also in p110 $\delta$  but not in p110 $\alpha$  [45]. This indicates that p85 $\alpha$  and most likely other p85 isoforms have unique regulatory interactions with different class IA p110 isoforms. As the development of p110 isoform specific inhibitors emerge, identification of genomic markers that predict the effect of different p85 $\alpha$  mutations on response will be important for successful application of the isoform-selective therapeutic strategies in the clinic. Moreover, it would be interesting to investigate whether p110 mutants that disrupt the p85 $\alpha$  inhibitory contact, such as p110 $\alpha$ -N345, confer similar sensitivity to the same group of inhibitors as these hypomorphic iSH2 p85 $\alpha$  mutants. Further, p110 isoforms have been shown to contribute to blood platelet activation which is essential for coagulation [54]. If p110 isoform specific inhibitors are given to mutant-carrying cancer patients,



bleeding risk upon blockage of platelet activation may have to be considered.

### Dominant negative & hypomorphic mutants that disrupt p85 $\alpha$ homodimerization &/or PTEN binding

Approximately a third of *PIK3R1* mutations are found outside the iSH2 domain. It is highly likely that these mutants are involved in altering p85 $\alpha$  functions independent of p110 because these N-terminal domains (see [Figure 1](#) for the domain structure of p85 $\alpha$ ) do not bind p110. Indeed, WT p85 $\alpha$  has demonstrated PI3K-independent activities by interaction with multiple signaling molecules through its N-terminal domains. For example, the BH domain of p85 $\alpha$  interacts with Cdc42 of the Rho family of small GTPases to regulate cytokinesis [55]. A direct interaction between the BH domain of p85 $\alpha$  and PTEN has also been demonstrated contributing to increased PTEN activity [56,57]. Thus p85 $\alpha$  has both positive (p110) and negative (PTEN) effects on the PI3K pathway allowing tight spatial and temporal regulation of pathway activity. The SH3 domain of p85 $\alpha$  binds to a proline-rich motif in the Na<sup>+</sup>, K<sup>+</sup>-ATPase  $\alpha$  subunit suppressing cell motility by reorganizing the actin cytoskeleton [58,59]. Intriguingly, p85 $\alpha$  itself has two proline-rich motifs and one of them contains a canonical class I SH3-interacting sequence, suggesting that there may be intramolecular or intermolecular interaction between the SH3 and the proline-rich domains of p85 $\alpha$  monomers or dimers, respectively. Moreover, p85 $\alpha$  is shown to be in excess of p110 $\alpha$  in fibroblasts [60], indicating that the amount of endogenous p85 $\alpha$  is more than p110 $\alpha$  in certain cell context and resulting in a pool of p85 $\alpha$  that is not bound to p110 $\alpha$ . p85 $\alpha$ , if in excess of p110 $\alpha$ , could compete with the p85 $\alpha$ -p110 $\alpha$  complex for binding to phosphorylated insulin receptor substrate or to other phosphotyrosine-containing proteins [61], suggesting that p110 $\alpha$ -unbound p85 $\alpha$  could act as a negative regulator of PI3K signaling and potentially act as a tumor suppressor.

We have recently proposed an alternative but compatible mechanistic model of how p110-free p85 $\alpha$  negatively regulates the PI3K pathway. p85 $\alpha$  homodimers were first reported in 1999 [62]. We have extended this finding by demonstrating that p110 $\alpha$ -unbound p85 $\alpha$  homodimerizes through multiple intermolecular interactions between two p85 $\alpha$  monomers [37,63]. The SH3 domain of one p85 $\alpha$  protomer interacted with the first proline-rich motif (residues 93–99; proline-rich domain 1, [Figure 1](#)) of another p85 $\alpha$  protomer in a canonical SH3/proline-rich domain interaction, constituting a major homodimerization interface. Additional interactions through BH:BH domain

dimerization also contributed to stabilizing the homodimer. Intriguingly, the p85 $\alpha$  homodimer competed with the E3 ligase of PTEN WWP2 for binding to the PTEN phosphatase domain and protected PTEN against WWP2-mediated proteosomal degradation [63]. Further, p85 $\alpha$  homodimers also enhanced the lipid phosphatase activity and membrane association of PTEN providing increased access to its targets. Indeed, *Pik3r1* knockout mice display decreased PTEN protein levels and increased PI3K pathway activity [64]. p110 $\alpha$ -unbound homodimeric p85 $\alpha$ , therefore, indirectly downregulates PI3K signaling through positive regulation of PTEN.

Of significant clinical importance, we have identified cancer patient-derived p85 $\alpha$  mutants that disrupt p85 $\alpha$  homodimerization and PTEN binding resulting in decreased PTEN levels, indicating the relevance of the p85 $\alpha$  homodimer in cancer biology. One of these mutants E160\* leads to premature stop codon and a truncated protein lacking the BH domain and other C terminal domains. E160\* binds WT p85 $\alpha$  resulting in decreased WT p85 $\alpha$  dimers [37]. Consistent with the lack of the BH domain that binds PTEN, E160\* could not bind PTEN and further, the binding between PTEN and WT p85 $\alpha$  was decreased in the presence of E160\* [37]. E160\*, therefore, represents a dominant negative mutant inhibiting p85 $\alpha$  homodimerization. As a result, WT p85 $\alpha$  does not interact with PTEN and does not protect PTEN from ubiquitination. I177, which is located at the BH:BH p85 $\alpha$  homodimer interface, is highly conserved across species. Intriguingly, a naturally occurring I177N mutation decreased homodimer formation and decreased PTEN binding [63] with a consequent decreased PTEN protein levels and increased phosphorylated AKT. Thus I177N is a hypomorph due to weakened ability to form homodimers and a decreased ability to stabilize PTEN compared with WT p85 $\alpha$ . Strikingly, another cancer patient-derived p85 $\alpha$  point mutation hypomorph located at the PTEN interacting interface has been discovered. Without an impact on p85 $\alpha$  homodimer formation, the mutant I133N led to decreased PTEN binding, increase in PTEN ubiquitination and activation of the PI3K pathway [63]. There are multiple other patient mutations in regions potentially involved in p85 $\alpha$  homodimerization [37,40] for example, other truncation mutants around E160 ([Figure 1](#)). It is very likely that these mutants share the same oncogenic mechanism as E160\*. The proline residue (residue 99) in the first proline-rich motif, which is involved in p85 $\alpha$  homodimerization, is a site of recurrent mutation.

Similar to the mutants within the SH2 domains described above, these p85 $\alpha$  homodimer and/or PTEN destabilizing mutants lead to activation of

the downstream PI3K pathway, therefore implicating PI3K pathway inhibitors as potential therapies for tumor cells carrying these mutants. Indeed, a drug sensitivity screen with a 145-compound library revealed that E160\* rendered BaF/3 cells more sensitive to mTOR inhibitors (rapamycin and temsirolimus), but not to pan-PI3K inhibitors (GSK2126458A and GDC0941) [65]. The cells were not sensitive to RTK inhibitors such as the EGFR inhibitors lapatinib and erlotinib [65]. Observations from studies by others suggest that PI3K pathway activation confers resistance to RTK inhibition and that dual blockade of PI3K pathway and RTK may improve the therapeutic outcome [66–68]. Interestingly, along this line, E160\* conferred BaF/3 cells sensitivity to the inhibitor PP121 which is a dual inhibitor of RTKs (Abl, Src, VEGFR–2 and PDGFR) and PI3K family kinases (p110 $\alpha$ , DNA-PK and mTOR). The efficacy of such combined inhibition definitely needs further confirmation using more inhibitors in more cell models.

A previous study has shown that subtle decreases in PTEN expression may have profound effects in promoting tumor-suppressor ‘quasi-insufficiency’ and increased susceptibility to cancer [69]. With the discovery of PTEN-destabilizing p85 $\alpha$  mutants, it may be important to investigate whether cells with the PTEN-destabilizing p85 $\alpha$  mutants display the same drug sensitivity profile as PTEN-deficient cells. The preclinical and clinical drug sensitivity data of PTEN-deficient cancer cells available so far are conflicting, for example, to AKT inhibitor [70,71] or mTOR inhibitor [72–74]. This is probably due to the differences in genetic background in these cell models under investigation with an isogenic cell panel potentially providing a better model for the comparison. Further, p110 $\beta$ , but not p110 $\alpha$ , is suggested to be the dominant PI3K isoform mediating PI3K signaling in PTEN-deficient tumors [75,76]. Some studies demonstrated that the p110 $\beta$ -selective inhibitor was more effective than p110 $\alpha$ -specific inhibitors in PTEN-deficient tumors in at least some lineages [75,77–79]. The therapeutic liability engendered by the PTEN-destabilizing mutants to PI3K isoform specific inhibitors would be another interesting area for investigation.

### Neomorphic mutants that activate the MAPK signaling pathways

Perhaps the most exciting discovery among all functionally characterized p85 $\alpha$  mutants is the identification of a neomorph that activates the MAPK pathway in addition to the PI3K pathway. R348\* is the most recurrent *PIK3R1* mutation in endometrial cancers (9.6 and 6.9% of all *PIK3R1* mutations in our and TCGA datasets, respectively) and colon cancers

(13.3%; TCGA) [37,40]. Multiple patient-derived truncation mutations within close distance from R348 have also been detected including a Q329fs in renal papillary cell carcinoma and a L370fs in ovarian cancer [40] (Figure 1). This high occurrence frequency provides justification for investigating whether these mutations can be biomarker of drug response and the underlying mechanism of sensitivity.

Consistent with an activation of AKT, R348\* engendered sensitivity to an AKT inhibitor MK2206 [65]. In contrast to E160\*, R348\* had no effect on sensitivity to rapamycin. While both E160\* and R348\* do not stabilize the PTEN protein, the activation of signaling downstream of AKT (including phosphorylation of S6, p70SK6 and GSK3) was more robust in cells with R348\* than E160\* mutations [65]. The distinct drug sensitivity profiles could, therefore, be due to difference in activation mechanisms or degree of activation of the pathway and/or involvement of different feedback machinery along the PI3K pathway axis. The exact mechanism by which R348\* activates the PI3K pathway warrants further investigation. Preliminary data suggested that the activation requires the activities of Cdc42 and Rac1 [80].

Surprisingly, BaF/3, endometrial or ovarian cancer cells expressing R348\* or L370fs displayed sensitivity to multiple MEK (PD0325901, AZD6244, PD98059, CI1040 and hypothemycin) and JNK (SP600125, BI78D3 and AEG3482) inhibitors [65]. The sensitivity to these MAPK inhibitors was unique to R348\*- or L370fs-expressing cells because WT p85 $\alpha$ , E160\* and other known p85 $\alpha$  driver mutants (DKRMNS-560del, R574fs and T576del) had no effect on sensitivity towards these inhibitors, highlighting the neomorphic activities of R348\* and L370fs [65]. Xenograft models with endometrial or ovarian cancer cells stably expressing the neomorphs displayed significantly increased tumor growth and the tumors were highly sensitive to MEK and JNK inhibitors. Activation of the pathways appears to underlie pattern of sensitivity to the inhibitors because the neomorphs were associated with phosphorylation of components along the ERK and JNK pathways. Higher phosphorylation of nuclear ERK and JNK could also be observed by immunohistochemical staining in endometrial cancer patients with R348\* compared with those without the mutation [65]. Importantly, the activation of MAPK by the neomorphs was independent of the convention role of p85 $\alpha$  in PI3K signaling and this ties in perfectly to the fact that R348\* lacks an iSH2 domain that mediates association with p110. The neomorphic activity is expected to be applicable to multiple patient-derived truncating and other mutations in *PIK3R1* within close proximity of R348 or L370 (Figure 1).

The ability of the neomorphs to activate the MAPK pathways underscores the need to functionally characterize each patient's specific driver mutation. The identification of neomorphs argues that patient stratification for treatment approaches needs to be based on the specific mutations present in the cancer gene rather than on the mutated cancer gene alone because of the potential unique functional consequences of individual mutations. The treatment strategy for tumors with the p85 $\alpha$  neomorphs may be similar to that of *KRAS* driven tumors or tumors with co-mutations in both MAPK and PI3K pathways. Activated MAPK pathway in Ras mutant tumors mediates resistance to therapy targeting PI3K pathway [81]. Conversely, PI3K pathway has also been implicated in mediating resistance to MEK inhibitors [82,83]. Combined inhibition of PI3K and MAPK signaling may be required to achieve maximal therapeutic response.

### Reduced *PIK3R1* expression also confers drug sensitivity

*PIK3R1* was significantly decreased in a subset of human cancer tissues including ovarian, prostate, liver, lung, breast, kidney, compared with control tissues [64,84]. Reduced expression of *PIK3R1* mRNA and p85 $\alpha$  protein was associated with poorer metastasis-free survival of breast cancer patients [84]. Further, low *PIK3R1* mRNA level was a signature of high-risk stage I nonsmall cell lung cancers compared with their low-risk counterparts [85]. Likewise, reduced *PIK3R1* mRNA expression was associated with migration and invasion of breast cancer cell line [86]. In mouse models of hepatocellular cancer, liver specific-*Pik3r1* knockout with 80–90% decrease in p85 $\alpha$  protein expression led to PI3K pathway activation and conferred spontaneous tumor development [64]. Consistent with a role of p85 $\alpha$  in PTEN stabilization, these *Pik3r1* knockout tumors had decreased PTEN expression. All these findings clearly suggest that reduced p85 $\alpha$  expression promotes tumorigenesis at least in certain tumor types. One plausible underlying mechanism could be decrease in the amount of p110–unbound p85 $\alpha$ , which is tumor suppressive as described earlier. However, it is important to note that *in vitro* knockdown of *PIK3R1* resulted in decreased proliferation, migration and invasion in glioblastoma multiforme cells [87], suggesting that increased or decreased p85 $\alpha$  levels could have similar effects on tumor progression depending on the context.

Loss-of-function RNAi-based screen showed that silencing *PIK3R1* enhanced the sensitivity of breast cancer cell lines to rapamycin [88], implicating a negative role of *PIK3R1* in PI3K pathway activation. Intriguingly, the histone deacetylase inhibitor (HDACi)

decreased the expression of *PIK3R1* mRNA, suggesting the use of combination of HDACi and rapamycin as a potential treatment. The rapamycin/HDACi combination led to decreased PI3K pathway signaling, induced G<sub>0</sub>/G<sub>1</sub> cell cycle arrest as well as enhanced cleavage of PARP and caspase-3 [88]. As another level of evidence, the effect of the rapamycin/HDACi combination of growth of breast cancer cells injected into the mammary fat pad of female SCID mice was associated with decreased p85 $\alpha$  protein expression and PI3K pathway inhibition [88]. Another independent study showed that *PIK3R1* was overexpressed in cisplatin-resistant ovarian cancer cell lines and knockdown of *PIK3R1* resensitized these cell lines to platinum treatment, indicating the potential involvement of p85 $\alpha$  in acquired cisplatin resistance [89]. The PI3K pathway has been shown to be a mediator of platinum resistance [90–92]. For example, AKT activation conferred resistance to caspase-independent cisplatin-induced apoptosis by inhibiting the apoptosis-inducing factor-associated pathway [91]. However, the mechanism of how p85 $\alpha$  mediates resistance to chemotherapy and whether this involves the canonical PI3K signaling pathway remain to be investigated.

### Conclusion

Different driver aberrations of the same gene may be functional through divergent oncogenic mechanisms and influence distinct downstream signaling processes. *PIK3R1* can be hailed as the 'poster child' for this concept. Although the p85 $\alpha$  driver mutants characterized thus far all activate the PI3K pathway, the mutants may lead to activation of different nodes along the axis or elicit different feedback mechanisms therefore engendering sensitivity to inhibitors of different nodes in the pathway. The mutants may also activate unpredicted pathways, for example the MAPK pathway. Clearly, tailored therapeutic approaches will be required to target these individual *PIK3R1* mutations.

### Future perspectives

The key challenge for successful implementation of targeted therapy is to identify the appropriate patient populations most likely to benefit from the treatment. Currently, many targeted therapeutics are entering clinical evaluation and even more compounds are under development. The development and validation of biomarkers that can identify the sensitive or resistant populations appears to be much slower than the pace of new drug development. Indeed, only a small subset of targeted cancer therapies are administered based on the genetic background of the tumors [93–95]. Aggressive efforts in functional characterization of drivers are needed to speed up the translation of genomic data into

the clinic, in order to identify additional biomarkers and to devise better therapeutic strategies.

The *PIK3R1* gene is currently included in the sequencing panel of multiple clinical laboratories for the analysis of cancer patient tumor samples, further highlighting the importance of understanding of the biological significance of the genomic alterations of the gene. In addition to small molecule inhibitors that target specific kinases along the PI3K pathway, the identification of interacting partners of p85 $\alpha$  and cancer patient-derived mutants that target these interactions may allow development of potential alternative treatment approaches. For instance, pharmacological compounds or small peptides that specifically target

the interacting interfaces between p85 $\alpha$  and p110 or PTEN may be a new avenue of targeting tumors harboring specific *PIK3R1* mutations.

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#### Executive summary

- Functionally characterized p85 $\alpha$  mutants are hypomorphs, dominant negative mutants or neomorphs.
- *PIK3R1* aberrations including gene mutations or downregulation of gene expression could potentially be a biomarker of responsiveness to inhibitors targeting different nodes along the PI3K pathway or the MAPK pathway.
- Detailed functional characterization and identification of underlying mechanism of driver mutations is imperative to deliver optimal therapy to cancer patients.

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