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## Targeting the protein-protein interaction between IRS1 and mutant p110 $\alpha$ for cancer therapy

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### Abstract

*PIK3CA*, which encodes the catalytic p110 $\alpha$  subunit of phosphatidylinositol 3-kinase  $\alpha$ , is the most frequently mutated oncogene in human cancers. Targeting mutant p110 $\alpha$  holds great promise for cancer therapy. However, it is challenging to develop p110 $\alpha$  isoform-specific inhibitors. Most p110 $\alpha$  mutations occur at two hotspot regions: an acidic cluster (E542, E545 and Q546) in the helical domain and a histidine residue (H1047) in the kinase domain. We recently discovered that p110 $\alpha$  helical domain mutant proteins, but not the kinase domain mutant proteins, directly associate with IRS1. Moreover, we demonstrated that disruption of protein-protein interaction between p110 $\alpha$  helical domain mutant and IRS1 inhibits the growth of tumors with such mutations. The direct protein interaction between IRS1 and p110 $\alpha$  helical domain mutants may provide a more accessible target for developing novel precision cancer therapy.

### Keywords

Cancer; Molecular biology; Genomics

### Introduction

Targeted cancer therapy is transforming the way that doctors treat cancer patients. In contrast to standard chemotherapies that indiscriminately kill rapidly dividing cells in human body, targeted cancer therapies are designed to affect specific molecules that are part of the pathways and processes used by cancer cells to grow, divide, and metastasize (Gschwind *et al.*, 2004). A major advantage of targeted therapies is that they elicit fewer toxic side effects than standard chemotherapies because they often cause little or no damage to normal cells. The best targets for cancer therapies are these molecules or pathways that are only present in cancer cells but not in normal cells (Gschwind *et al.*, 2004). For example, genetically altered proteins resulting from somatic mutations and translocations are ideal targets. A paradigm of targeted cancer therapy is the successful treatment of chronic myelogenous leukemia (CML) with Imatinib (branded as Gleevec) (Druker *et al.*, 2001). Over 90% of CML patients harbor a reciprocal translocation between chromosome 9 and 22, which results in an in-frame gene fusion between BCR and ABL1 gene. Imatinib inhibits the tyrosine kinase activity of BCR-

Abl fusion protein, thereby killing only CML cells but not normal cells (Druker *et al.*, 2001). Recently, the FDA approved Vemurafenib, a small molecule that inhibits mutant B-raf kinase activity, for treating metastatic melanoma patients with a BRAF V600E mutation. This success highlights the importance of targeting mutant oncoproteins for cancer therapy (Sosman *et al.*, 2012). Given that recent cancer genome sequencing studies reveal that *PIK3CA* is the most frequently mutated oncogene in human cancers (Garraway and Lander, 2013), the mutant *PIK3CA* gene product, p110 $\alpha$ , is an important target for cancer therapy. Here we review the promises and challenges of targeting mutant p110 $\alpha$ .

## The PI3K/AKT pathway is a central intracellular signaling pathway

Phosphatidylinositol 3-kinases (PI3Ks) play key roles in regulating cell proliferation, survival and motility (Figure 1). The PI3K family proteins are classified into three groups (see more detailed discussion below). The class I PI3Ks consist of a catalytic p110 subunit and a regulatory p85 subunits. In the basal state, the regulatory p85 subunit stabilizes the catalytic p110 subunit and inhibits its enzymatic activity (Liu *et al.*, 2009). Upon growth factor stimulation, the SH2 domains of p85 bind to the phospho-tyrosine residues on the receptor protein kinases or adaptor proteins such as insulin receptor substrate 1 (IRS1), thereby activating the lipid kinase activity of PI3Ks (Cantley, 2002). Activated PI3Ks convert phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>) to phosphatidylinositol-3,4,5-triphosphate (PIP<sub>3</sub>). PIP<sub>3</sub> then recruits proteins that contain a pleckstrin homology domain (PH domain) including AKT serine/threonine kinase and 3-phosphoinositide-dependent kinase -1 (PDK1) to the cell membrane (Cantley, 2002). The active membrane associated AKT and PDK1 phosphorylate numerous protein targets including MDM2, GSK3 $\beta$ , IKK, tubrin and a subset of forkhead transcription factors (Cantley, 2002). The tumor suppressor gene Phosphatase and Tensin homolog deleted on chromosome Ten (PTEN) catalyzes the dephosphorylation of PIP<sub>3</sub> to generate PIP<sub>2</sub>, thereby negatively regulating the PI3K signaling pathways (Li *et al.*, 1997). Inactivating mutations of PTEN have been found to occur in several different tumor types (Li *et al.*, 1997).

## The discovery of *PIK3CA*/p110 $\alpha$ mutation in human cancers provides an excellent target for cancer therapy

In the mutational analysis of PI3K gene family, Samuel *et al.* discovered almost a decade ago that *PIK3CA* (encoding p110 $\alpha$ ) was mutated in a variety of human cancers including brain, breast, colon, gastric and lung cancers (Samuels *et al.*, 2004). Follow-up studies by many laboratories and recent comprehensive cancer genome sequencing of a large collection of various human cancers by The Cancer Genome Atlas (TCGA) project revealed that *PIK3CA* is the most frequently mutated oncogene in human cancers (Table 1) (Bachman *et al.*, 2004, Broderick *et al.*, 2004, Campbell *et al.*, 2004, Lee *et al.*, 2005, Levine *et al.*, 2005, Li *et al.*, 2005, Oda *et al.*, 2005, Garraway and Lander, 2013). Interestingly, nearly all of the mutations are heterozygous and the majority are clustered in at two hotspot regions: an acidic cluster (E542, E545 and Q546) in the helical domain and a histidine residue (H1047) in the kinase domain (Figure 2). Results from *in vitro* lipid kinase assays indicated that the hotspot mutations, H1047R and E545K, resulted in increased lipid kinase activity, suggesting that the mutant p110 $\alpha$  indeed acts as an oncogene (Samuels *et al.*, 2004). Targeted inactivation of the *PIK3CA* oncogenic mutations in human colorectal cancer cell lines showed that oncogenic alleles of *PIK3CA* render cancer cells growth advantage in low serum condition, attenuation of apoptosis and facilitating tumor invasion (Samuels *et al.*, 2005). Interestingly, mutant p110 $\alpha$  appears to selectively activate AKT1 and the forkhead transcription factors FKHR and FKHL1 (Samuels *et al.*, 2005). Given that the oncogenic mutant p110 $\alpha$  is a lipid kinase, it is a very promising drug target for cancer therapy as it is actionable, but a potential limitation is specificity.

## Challenges of targeting mutant p110 $\alpha$

Although scientists at both pharmaceutical companies and academic institutions have made enormous effort to develop drugs to inhibit mutant p110 $\alpha$  enzymatic activity, it turns out that it is difficult to find small molecules that specifically inhibit p110 isoforms (Liu *et al.*, 2009). Based on the substrate specificities and structural features, PI3K family members are divided into three classes (Figure 3). Class I PI3Ks are heterodimers consisting of a p110 catalytic subunit and a p85 regulatory subunit. The p85 regulatory subunit modulates the activity, stability and localization of the enzyme. Three genes *PIK3R1*, *PIK3R2* and *PIK3R3* encode p85 $\alpha$  (and its splice variants p55 $\alpha$  and p50 $\alpha$ ), p85 $\beta$  and p55 $\gamma$  respectively. All these regulatory subunits are collectively referred as p85. The class I PI3Ks convert PIP2 to PIP3. Both Class II and class III PI3K family members have only a single catalytic subunit. The class II PI3Ks (PIKC2 $\alpha$ , PIKC2 $\beta$  and PIKC2 $\gamma$ ) use phosphatidylinositol or phosphatidylinositol-4-phosphate as substrates; whereas the single class III PI3K member, VPS34, converts phosphatidylinositol to phosphatidylinositol-3-phosphate.

The class I PI3Ks have been intensively characterized. This group of PI3Ks is further divided into class IA and class IB subgroups. The Class IA PI3Ks have three members: PI3K $\alpha$ , PI3K $\beta$  and PI3K $\delta$ . The catalytic subunits of these three enzymes share similar domain structures: an N-terminal adaptor-binding domain (ABD, also called p85 binding domain), a Ras-binding domain (RBD), a C2 domain, a helical domain and a catalytic domain (Figure 3). The p110 catalytic subunit of the class IA PI3Ks form a heterodimer with one of the p85 regulatory subunits (p85 $\alpha$ , p85 $\beta$ , p55 $\alpha$ , p55 $\gamma$  and p50 $\alpha$ ). The class IB PI3K consists of the catalytic subunit p110 $\gamma$  and regulatory subunit p101 (Figure 3). The overall domain structure of p110 $\gamma$  is similar to these of PI3K $\alpha$ , PI3K $\beta$  and PI3K $\delta$ , but it lacks the ABD domain (Figure 3). While p110 $\alpha$  and p110 $\beta$  are ubiquitously expressed, p110 $\delta$  and p110 $\gamma$  are mainly expressed in leukocytes (Liu *et al.*, 2009). Interestingly, only p110 $\alpha$  is mutated in human cancers (Zhao and Vogt, 2008a). Recent crystal structure analyses indicate that the structures of class I PI3K catalytic domains are quite similar and that the ATP binding pockets of these enzymes are almost identical (Vadas *et al.*, 2011), which may explain why it is difficult to develop p110 $\alpha$  isoform-specific inhibitors.

Currently, several pan-class I PI3K inhibitors (GDC-0941, BKM120, PX866 and BAY 80-6946) and dual PI3K/mTOR inhibitors (e. g. BEZ235) are in early-stage clinical trials (Rodon *et al.*, 2013). There are also some PI3K $\beta$ -specific and PI3K $\delta$ -specific inhibitors under clinical evaluation for their efficacy against various tumor types (Rodon *et al.*, 2013). It is worth noting that Genentech has recently developed a so-called  $\beta$ -spare PI3K inhibitor GDC-0032, which selectively inhibits p110 $\alpha$ , p110 $\delta$  and p110 $\gamma$ , but sparing the p110 $\beta$  isoform. Early-stage clinical trials in breast cancer patients has shown great promise. Patients harboring PIK3CA/p110 $\alpha$  had at least partial clinical responses to the  $\beta$ -spare PI3K inhibitor (Juric D, personal communication), suggesting that a p110 $\alpha$  isoform-specific inhibitor should be more potent and less toxic. Nonetheless, drugs that specifically target the mutant p110 $\alpha$  should be highly advantageous.

## Mutant-specific protein interaction between IRS1 and helical domain mutations of p110 $\alpha$ may provide a more accessible target for cancer therapy

Given the challenges encountered with development of p110 $\alpha$ -specific inhibitors, alternative approaches are needed to target mutant p110 $\alpha$  for cancer therapy. Our recent discovery that the helical domain p110 $\alpha$  mutants directly interact with IRS1 may provide a more accessible target for developing drugs to treat cancer patients harboring those p110 $\alpha$  mutations (Hao *et*

*et al.*, 2013). We discuss below the relevant background and therapeutic implications of our findings.

1. The p110 $\alpha$  helical domain and kinase domain mutations exert their oncogenic functions through distinct mechanisms.

As mentioned above, most of p110 $\alpha$  mutations occur at two hotspot regions: an acidic cluster (E542, E545 and Q546) in the helical domain and a histidine residue (H1047) in the kinase domain. The E545K and H1047R are the two most frequently observed p110 $\alpha$  somatic mutations in human cancers. Interestingly, several recent studies indicate that the E545K and H1047R mutations exert their oncogenic functions through distinct mechanisms: (1) Zhao and Vogt have shown that the helical domain and the kinase domain mutant proteins could synergistically transform chicken embryonic fibroblasts (Zhao and Vogt, 2008b), providing the first clue that the two mutations may induce oncogenic transformation through different pathways. Consistently, they further demonstrated that the p110 $\alpha$  helical domain mutants require the Ras-binding domain for their transformation activities, whereas the oncogenic activity of the H1047R mutant depends on the ABD domain. (2) Pang *et al.* showed that expression of the p110 $\alpha$  E545K mutant produce a more severe metastatic phenotype than that of the H1047R mutant in a breast cancer cell line (Pang *et al.*, 2009). (3) In contrast, the p110 $\alpha$  H1047R mutant, but not the E545K mutant, is found to enhance HER2-mediated transformation of immortalized mammary epithelial cells (Chakrabarty *et al.*, 2010). Structural analysis indicates that the p110 $\alpha$  H1047R mutation alters the interaction between PI3Ka and the cell membrane, thereby activating its kinase activity (Mandelker *et al.*, 2009). It has been suggested that the helical domain mutations activate their enzymatic activities by disrupting the inhibitory effect of the p85 subunits (Huang *et al.*, 2007, Miled *et al.*, 2007).

2. *Direct interaction between p110 $\alpha$  helical domain mutants and IRS1 drives tumorigenesis.* Our recent studies however indicate that the weakened p110 $\alpha$ -p85 interaction caused by the p110 $\alpha$  helical domain mutations is not sufficient for these mutant proteins to exert their oncogenic functions. In an attempt to determine how p110 $\alpha$  E545K and p110 $\alpha$  H1047R differentially activate oncogenic signaling pathways, we tried to identify proteins that might differentially bind to wild-type (WT) and mutant p110 $\alpha$ . To this end, we discovered that IRS1 binds to p110 $\alpha$  E545K mutant but not to WT or H1047R mutant under serum-starvation conditions (Hao *et al.*, 2013). In addition, other hotspot p110 $\alpha$  mutations in the helical domain including E542K, E545A, E545G and Q546K as well as relatively rare mutations including K111N in the ABD domain and N345K in the C2 domain also gain interaction with IRS1 under serum-starvation conditions (Hao *et al.*, 2013). We provided compelling evidence that the p110 $\alpha$  E545K mutant-IRS1 interaction plays a critical role in tumorigenesis and demonstrated how this mutant-specific protein interaction may be exploited for cancer therapy. Firstly, in the IRS1 KO DLD1 cells harboring the p110 $\alpha$  E545K mutation, reconstitution of an IRS1 deletion construct that does not interact with p110 $\alpha$  E545K mutant protein produces smaller xenograft tumors compared to the full-length IRS1 reconstituted cells (Hao *et al.*, 2013). Secondly, a p110 $\alpha$  E545K mutant stapled peptide, but not the WT counterpart, specifically inhibits xenograft tumor growth of CRC cells with a p110 $\alpha$  E545K mutation (Hao *et al.*, 2013). Mechanistically, our data suggest that p110 $\alpha$  E545K mutant-IRS1 interaction activates the mutant p110 $\alpha$  lipid kinase in two ways: (1) it brings the mutant enzyme complexes from cytosol to plasma membrane; (2) it stabilizes the mutant p110 $\alpha$  proteins (Hao *et al.*, 2013). We propose that the helical domain mutations of p110 $\alpha$ , as well as some of the

mutations in the ABD and C2 domain, induce conformational changes that enable p110 $\alpha$  to directly interact with IRS1, which does not require IRS1 tyrosine phosphorylation and p85 proteins (Figure 4). In support, a recent study shows that oncogenic mutations of p110 $\alpha$  in various domains indeed induce conformational changes of its protein structure that may affect its structural interactions (Burke *et al.*, 2012).

Our studies may provide an explanation for observed different phenotypes produced by the helical and kinase domain mutations. Using isogenic breast cancer cell lines, Pang and colleagues demonstrated that expression of p110 $\alpha$  helical domain mutations increase the sensitivity of cancer cells to chemo-attractants and induce much stronger metastatic phenotypes than the H1047R kinase domain mutation (Pang *et al.*, 2009). These phenotypic differences cannot be simply explained by the increased enzymatic activities of the helical and kinase domain mutations. In fact, Vasudevan *et al.* observed that the basal levels of phospho-AKT are lower in cancer cell lines expressing p110 $\alpha$  helical domain mutations compared to these cell lines expressing the H1047R kinase domain mutation (Vasudevan *et al.*, 2009). Consistently, when complexed with p85 regulatory subunit, the p110 $\alpha$  H1047R kinase domain mutant protein displays higher lipid kinase activity *in vitro* than the E545K helical domain mutant (Samuels *et al.*, 2005). We showed that the p110 $\alpha$  helical domain mutants, but not the kinase domain mutant, directly associate with IRS1 without growth factor stimulation (Hao *et al.*, 2013). In this regard, we suggest that the p110 $\alpha$  helical domain mutant-IRS1 protein complexes may contain additional proteins that do not exist in the p110 $\alpha$  kinase domain mutant protein complexes, thereby producing distinct phenotypes.

3. The protein interaction between IRS1 and p110 $\alpha$  helical domain mutants is independent of the p85 regulatory subunit.

Given that p110 $\alpha$  is brought to the IRS1 complex through interaction between phospho-IRS1 and p85 regulatory subunit when cells are stimulated with growth factor, it is important to demonstrate that the p110 $\alpha$  helical domain mutant-IRS1 interaction is independent of p85. Our studies provided several lines of evidence indicating that interaction between the p110 $\alpha$  helical domain mutant proteins and IRS1 is not mediated by binding of p85 SH2 domain with pY residues on IRS1. Firstly, p110 $\alpha$  helical domain mutant-IRS1 interaction is independent of IRS1 tyrosine phosphorylation (Hao *et al.*, 2013). Secondly, a p110 $\alpha$  E545K mutant protein (p110 $\alpha$  E545K $\Delta$ ABD) devoid of the ability to bind with p85 proteins still interacts with IRS1 (Hao *et al.*, 2013). This result also provides an explanation for the observation made by Vogt and colleagues that the helical domain mutants of p110 $\alpha$  do not require the binding of p85 to transform chicken embryonic fibroblasts (Zhao and Vogt, 2008b). Thirdly, we show here that ablation of p85 proteins does not reduce the p110 $\alpha$  E545K mutant-IRS1 interaction (Hao *et al.*, 2013).

Interestingly, diminishment of p85 proteins actually enhances the p110 $\alpha$  E545K mutant-IRS1 interaction, suggesting that p85 may compete with IRS1 to bind to the p110 $\alpha$  helical mutants (Hao *et al.*, 2013). Fourthly, in the absence of p85 proteins, recombinant IRS1 proteins bind *in vitro* to p110 $\alpha$  E545K mutant but not WT p110 $\alpha$  proteins (Hao *et al.*, 2013). Fifthly, an IRS1 deletion that abrogates IRS1 interaction with p110 $\alpha$  E545K mutant still binds well with p85 proteins (Hao *et al.*, 2013). Sixthly, a p110 $\alpha$  E545K mutant peptide, which disrupts the p110 $\alpha$  E545K mutant-IRS1 interaction, does not affect the binding of the mutant p110 $\alpha$  to p85 (Hao *et al.*, 2013). However, we point out that even though p85 proteins are not required for direct interaction between the p110 $\alpha$  helical domain mutants and IRS1, they may participate in an interaction that provides stability to the mutant because

ablation of p85 proteins results in significantly reduction of the levels of p110 $\alpha$  protein (Hao *et al.*, 2013).

4. Disrupting protein-protein interaction between p110 $\alpha$  helical domain mutant and IRS1 provide a novel approach for development of drugs targeting cancers with these mutations.

Increasing evidence suggests that protein-protein interactions are druggable targets (White *et al.*, 2008). In fact, small molecules that inhibit the MDM2-p53 interaction are being tested in clinical trials to treat cancer patients (Essmann and Schulze-Osthoff, 2011). Moreover, ABT-737, a small-molecule BH3-mimetic that inhibits protein-protein interaction of BCL2 family proteins, is also being evaluated in clinical trials for its efficacy in haematologic malignancies (Khaw *et al.*, 2011). Our observation that a p110 $\alpha$  E545K mutant staple peptide, but not the WT counterpart, specifically inhibits xenograft tumor growth of CRC cells with a p110 $\alpha$  E545K mutation provides a proof-of-principle for targeting this mutant specific protein interaction for cancer therapy. The discovery of frequent mutations of PIK3CA in human cancer provides a strong rationale for inhibition of mutated p110 $\alpha$  activities for targeted cancer therapy (Samuels *et al.*, 2005). However, as discussed above, it has remained a challenge to develop p110 $\alpha$  isoform-specific inhibitors. Our data suggest that disruption of the interactions between helical domain p110 $\alpha$  mutants and IRS1 may be exploited as a more accessible targeted therapy approach for cancer patients harboring such mutations. Given that this mutant p110 $\alpha$  specific interaction exists only in tumor cells that harbor these particular mutations, but not in normal tissues, drugs targeting this cancer-specific interaction should have no or minimal side effects because they do not perturb normal cellular functions. It is worth noting that our p110 $\alpha$  E545K mutant stapled peptide is not stable in blood stream, which prevents it being delivered systematically. A mean to improve the pharmacokinetics of the peptide is to substitute the peptide bonds with chemical bonds to generate a functional peptidomimetics. Alternatively, small molecules that can disrupt protein-protein interaction between IRS1 and p110 $\alpha$  helical domain mutant could be identified through high-throughput screening.

## Prospective

Mutant p110 $\alpha$  is excellent target for cancer therapy. Much of current effort has been devoted to develop p110 $\alpha$ -isoform specific inhibitors, which turns out to be a challenge task. Our recent finding that p110 $\alpha$  helical domain mutants gain direct interaction with IRS1 raises new hope of targeting this mutant specific protein interaction for cancer therapy. The key will be to screen or design small molecules with favorable pharmacokinetics that disrupt the protein-protein interaction between p110 $\alpha$  helical domain mutant and IRS1. In this regard, a co-crystal structure of p110 $\alpha$  helical domain mutant and IRS1 would be very helpful for both *in silico* design and optimization of compounds that disrupts the p110 $\alpha$  mutant-specific protein-protein interaction. Lastly, drugs targeting mutant p110 $\alpha$  may also be used to treat hemimegalencephaly, because recent next-gen sequencing analyses revealed that p110 $\alpha$  is also mutated in patients with this disease (Lee *et al.*, 2012).

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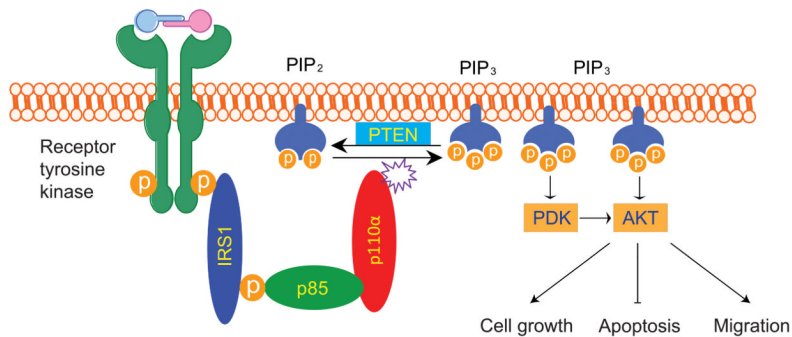
## References

- Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, Konishi H, Karakas B, Blair BG, Lin C, Peters BA, Velculescu VE, Park BH. The PIK3CA gene is mutated with high frequency in human breast cancers. *Cancer Biol Ther.* 2004; 3:772–775. [PubMed: 15254419]
- Broderick DK, Di C, Parrett TJ, Samuels YR, Cummins JM, McLendon RE, Fuhs DW, Velculescu VE, Bigner DD, Yan H. Mutations of PIK3CA in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas. *Cancer Res.* 2004; 64:5048–5050. [PubMed: 15289301]
- Burke JE, Perisic O, Masson GR, Vadas O, Williams RL. Oncogenic mutations mimic and enhance dynamic events in the natural activation of phosphoinositide 3-kinase p110alpha (PIK3CA). *Proc Natl Acad Sci U S A.* 2012; 109:15259–15264. [PubMed: 22949682]
- Campbell IG, Russell SE, Choong DY, Montgomery KG, Ciavarella ML, Hooi CS, Cristiano BE, Pearson RB, Phillips WA. Mutation of the PIK3CA gene in ovarian and breast cancer. *Cancer Res.* 2004; 64:7678–7681. [PubMed: 15520168]
- Cantley LC. The phosphoinositide 3-kinase pathway. *Science.* 2002; 296:1655–1657. [PubMed: 12040186]
- Chakrabarty A, Rexer BN, Wang SE, Cook RS, Engelman JA, Arteaga CL. H1047R phosphatidylinositol 3-kinase mutant enhances HER2-mediated transformation by heregulin production and activation of HER3. *Oncogene.* 2010; 29:5193–5203. [PubMed: 20581867]
- Colombino M, Sperlongano P, Izzo F, Tatangelo F, Botti G, Lombardi A, Accardo M, Tarantino L, Sordelli I, Agresti M, Abbruzzese A, Caraglia M, Palmieri G. BRAF and PIK3CA genes are somatically mutated in hepatocellular carcinoma among patients from South Italy. *Cell death & disease.* 2012; 3:e259. [PubMed: 22258409]
- Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med.* 2001; 344:1031–1037. [PubMed: 11287972]
- Dulak AM, Stojanov P, Peng S, Lawrence MS, Fox C, Stewart C, Bandla S, Imamura Y, Schumacher SE, Shefler E, McKenna A, Carter SL, Cibulskis K, Sivachenko A, Saksena G, Voet D, Ramos AH, Auclair D, Thompson K, Sougnez C, Onofrio RC, Guiducci C, Beroukhi R, Zhou Z, Lin L, Lin J, Reddy R, Chang A, Landrenau R, Pennathur A, Ogino S, Luketich JD, Golub TR, Gabriel SB, Lander ES, Beer DG, Godfrey TE, Getz G, Bass AJ. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. *Nat Genet.* 2013; 45:478–486. [PubMed: 23525077]
- Essmann F, Schulze-Osthoff K. Translational approaches targeting the p53 pathway for anticancer therapy. *Br J Pharmacol.* 2011
- Garcia-Dios DA, Lambrechts D, Coenegrachts L, Vandenput I, Capoen A, Webb PM, Ferguson K, Anecs, Akslen LA, Claes B, Vergote I, Moerman P, Van Robays J, Marcickiewicz J, Salvesen HB, Spurdle AB, Amant F. High-throughput interrogation of PIK3CA, PTEN, KRAS, FBXW7 and TP53 mutations in primary endometrial carcinoma. *Gynecologic oncology.* 2013; 128:327–334. [PubMed: 23219661]
- Garcia-Rostan G, Costa AM, Pereira-Castro I, Salvatore G, Hernandez R, Hermsem MJ, Herrero A, Fusco A, Cameselle-Teijeiro J, Santoro M. Mutation of the PIK3CA gene in anaplastic thyroid cancer. *Cancer Res.* 2005; 65:10199–10207. [PubMed: 16288007]
- Garraway LA, Lander ES. Lessons from the cancer genome. *Cell.* 2013; 153:17–37. [PubMed: 23540688]
- Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nat Rev Cancer.* 2004; 4:361–370. [PubMed: 15122207]
- Hao Y, Wang C, Cao B, Hirsch BM, Song J, Markowitz SD, Ewing RM, Sedwick D, Liu L, Zheng W, Wang Z. Gain of interaction with IRS1 by p110alpha-helical domain mutants is crucial for their oncogenic functions. *Cancer Cell.* 2013; 23:583–593. [PubMed: 23643389]
- Hartmann C, Bartels G, Gehlhaar C, Holtkamp N, von Deimling A. PIK3CA mutations in glioblastoma multiforme. *Acta neuropathologica.* 2005; 109:639–642. [PubMed: 15924253]

- Huang CH, Mandelker D, Schmidt-Kittler O, Samuels Y, Velculescu VE, Kinzler KW, Vogelstein B, Gabelli SB, Amzel LM. The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kalpha mutations. *Science*. 2007; 318:1744–1748. [PubMed: 18079394]
- Janku F, Lee JJ, Tsimberidou AM, Hong DS, Naing A, Falchook GS, Fu S, Luthra R, Garrido-Laguna I, Kurzrock R. PIK3CA mutations frequently coexist with RAS and BRAF mutations in patients with advanced cancers. *PLoS One*. 2011; 6:e22769. [PubMed: 21829508]
- Khaw SL, Huang DC, Roberts AW. Overcoming blocks in apoptosis with BH3-mimetic therapy in haematological malignancies. *Pathology*. 2011; 43:525–535. [PubMed: 21881535]
- Lee JH, Huynh M, Silhavy JL, Kim S, Dixon-Salazar T, Heiberg A, Scott E, Bafna V, Hill KJ, Collazo A, Funari V, Russ C, Gabriel SB, Mathern GW, Gleeson JG. De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. *Nat Genet*. 2012; 44:941–945. [PubMed: 22729223]
- Lee JW, Soung YH, Kim SY, Lee HW, Park WS, Nam SW, Kim SH, Lee JY, Yoo NJ, Lee SH. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene*. 2005; 24:1477–1480. [PubMed: 15608678]
- Levine DA, Bogomolny F, Yee CJ, Lash A, Barakat RR, Borgen PI, Boyd J. Frequent mutation of the PIK3CA gene in ovarian and breast cancers. *Clin Cancer Res*. 2005; 11:2875–2878. [PubMed: 15837735]
- Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliarensis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science*. 1997; 275:1943–1947. [PubMed: 9072974]
- Li VS, Wong CW, Chan TL, Chan AS, Zhao W, Chu KM, So S, Chen X, Yuen ST, Leung SY. Mutations of PIK3CA in gastric adenocarcinoma. *BMC Cancer*. 2005; 5:29. [PubMed: 15784156]
- Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov*. 2009; 8:627–644. [PubMed: 19644473]
- Mandelker D, Gabelli SB, Schmidt-Kittler O, Zhu J, Cheong I, Huang CH, Kinzler KW, Vogelstein B, Amzel LM. A frequent kinase domain mutation that changes the interaction between PI3Kalpha and the membrane. *Proc Natl Acad Sci U S A*. 2009; 106:16996–17001. [PubMed: 19805105]
- Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, Schneidman-Duhovny D, Wolfson HJ, Backer JM, Williams RL. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. *Science*. 2007; 317:239–242. [PubMed: 17626883]
- Oda K, Stokoe D, Taketani Y, McCormick F. High frequency of coexistent mutations of PIK3CA and PTEN genes in endometrial carcinoma. *Cancer Res*. 2005; 65:10669–10673. [PubMed: 16322209]
- Pang H, Flinn R, Patsialou A, Wyckoff J, Roussos ET, Wu H, Pozzuto M, Goswami S, Condeelis JS, Bresnick AR, Segall JE, Backer JM. Differential enhancement of breast cancer cell motility and metastasis by helical and kinase domain mutations of class IA phosphoinositide 3-kinase. *Cancer Res*. 2009; 69:8868–8876. [PubMed: 19903845]
- Phillips WA, Russell SE, Ciavarella ML, Choong DY, Montgomery KG, Smith K, Pearson RB, Thomas RJ, Campbell IG. Mutation analysis of PIK3CA and PIK3CB in esophageal cancer and Barrett's esophagus. *Int J Cancer*. 2006; 118:2644–2646. [PubMed: 16380997]
- Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat Rev Clin Oncol*. 2013; 10:143–153. [PubMed: 23400000]
- Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmstrom PO, Mansukhani M, Enoksson J, Hibshoosh H, Borg A, Parsons R. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res*. 2005; 65:2554–2559. [PubMed: 15805248]
- Samuels Y, Diaz LA Jr, Schmidt-Kittler O, Cummins JM, DeLong L, Cheong I, Rago C, Huso DL, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell*. 2005; 7:561–573. [PubMed: 15950905]
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE. High frequency of mutations of the PIK3CA gene in human cancers. *Science*. 2004; 304:554. [PubMed: 15016963]

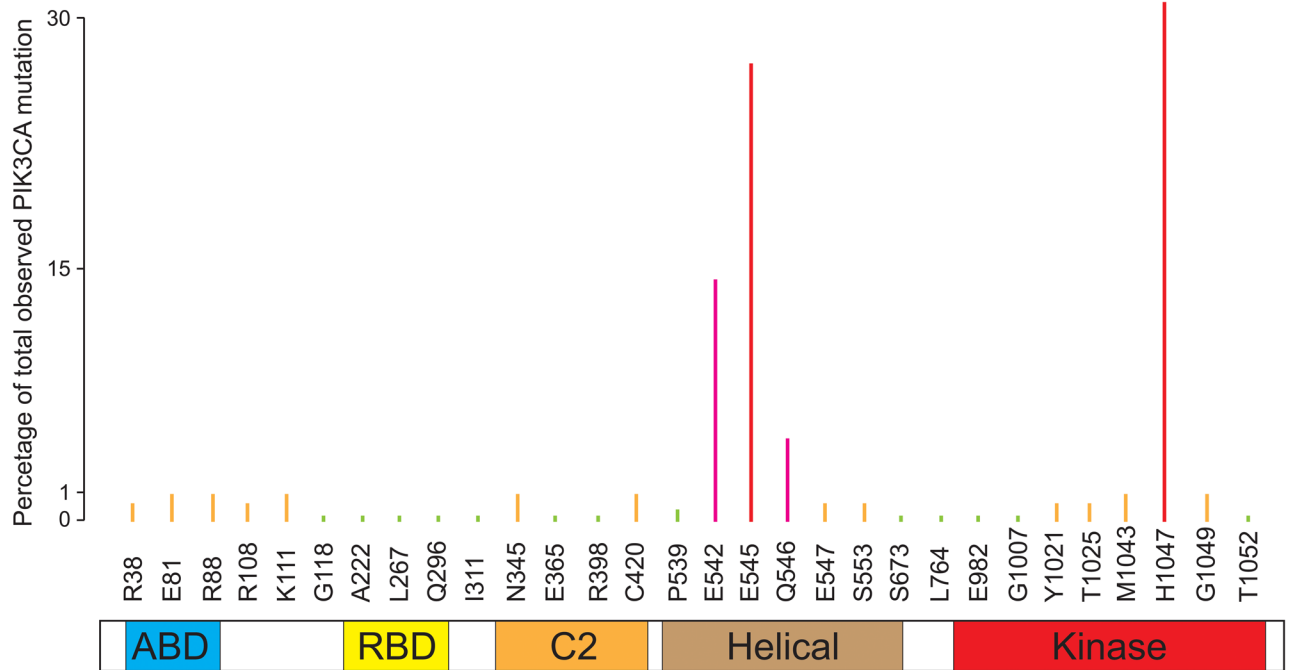


- Schonleben F, Qiu W, Ciau NT, Ho DJ, Li X, Allendorf JD, Remotti HE, Su GH. PIK3CA mutations in intraductal papillary mucinous neoplasm/carcinoma of the pancreas. *Clin Cancer Res.* 2006; 12:3851–3855. [PubMed: 16778113]
- Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, McArthur GA, Hutson TE, Moschos SJ, Flaherty KT, Hersey P, Kefford R, Lawrence D, Puzanov I, Lewis KD, Amaravadi RK, Chmielowski B, Lawrence HJ, Shyr Y, Ye F, Li J, Nolop KB, Lee RJ, Joe AK, Ribas A. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med.* 2012; 366:707–714. [PubMed: 22356324]
- Vadas O, Burke JE, Zhang X, Berndt A, Williams RL. Structural basis for activation and inhibition of class I phosphoinositide 3-kinases. *Sci Signal.* 2011;4. [PubMed: 21693763]
- Vasudevan KM, Barbie DA, Davies MA, Rabinovsky R, McNear CJ, Kim JJ, Hennessy BT, Tseng H, Pochanard P, Kim SY, Dunn IF, Schinzel AC, Sandy P, Hoersch S, Sheng Q, Gupta PB, Boehm JS, Reiling JH, Silver S, Lu Y, Stemke-Hale K, Dutta B, Joy C, Sahin AA, Gonzalez-Angulo AM, Lluch A, Rameh LE, Jacks T, Root DE, Lander ES, Mills GB, Hahn WC, Sellers WR, Garraway LA. AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. *Cancer Cell.* 2009; 16:21–32. [PubMed: 19573809]
- White AW, Westwell AD, Brahehi G. Protein-protein interactions as targets for small-molecule therapeutics in cancer. *Expert Rev Mol Med.* 2008; 10:e8. [PubMed: 18353193]
- Yamamoto H, Shigematsu H, Nomura M, Lockwood WW, Sato M, Okumura N, Soh J, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Date H, Lam WL, Minna JD, Gazdar AF. PIK3CA mutations and copy number gains in human lung cancers. *Cancer Res.* 2008; 68:6913–6921. [PubMed: 18757405]
- Zhao L, Vogt PK. Class I PI3K in oncogenic cellular transformation. *Oncogene.* 2008a; 27:5486–5496. [PubMed: 18794883]
- Zhao L, Vogt PK. Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci U S A.* 2008b; 105:2652–2657. [PubMed: 18268322]



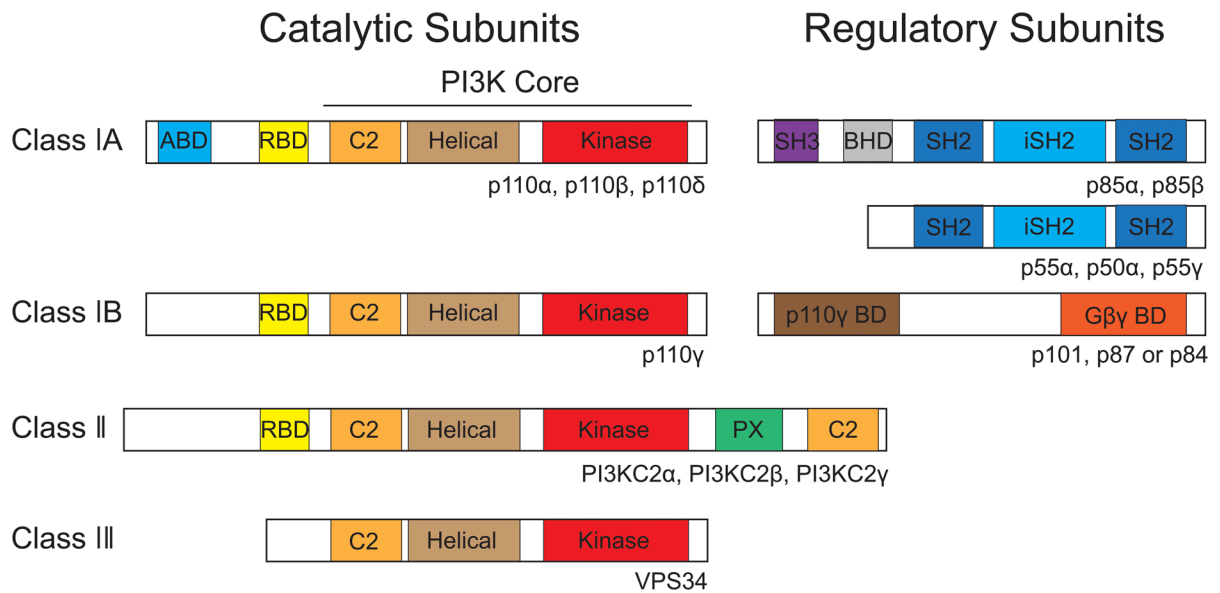
**Figure 1. The PI3K/AKT signaling pathway**

Growth factor stimulation leads to activation of receptor protein tyrosine kinases, which in turn phosphorylate adaptor proteins such as IRS1. Phospho-IRS1 then recruits the PI3K heterodimers (p85 regulatory and p110 catalytic subunits) and activates the lipid kinase activity. The activated PI3Ks convert PIP<sub>2</sub> to PIP<sub>3</sub>. The second messenger PIP<sub>3</sub> recruits PDK and AKT to cytoplasmic membrane, where PDK phosphorylates and activates AKT. Activated AKT phosphorylates an array of downstream targets that control cell proliferation, apoptosis and cell migration.



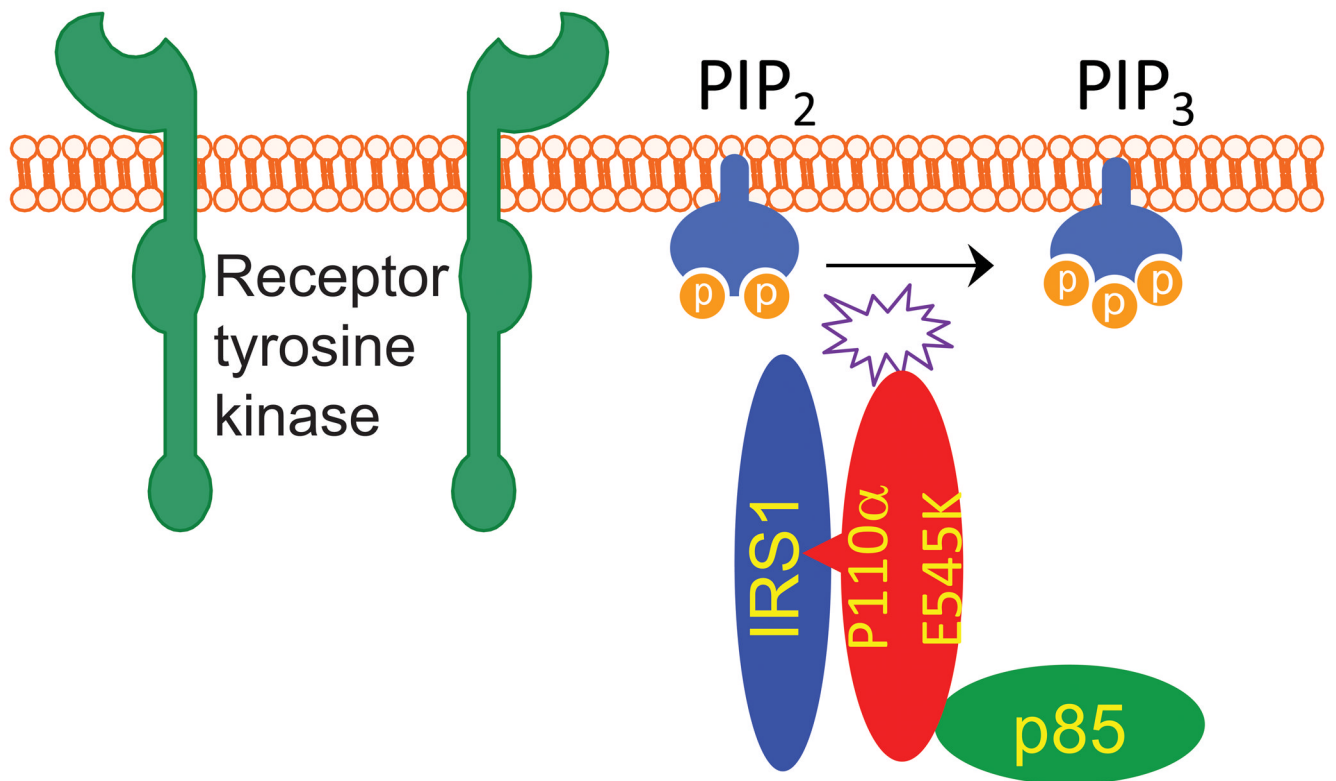
**Figure 2. Somatic mutation of PIK3CA/p110 $\alpha$  in human cancers**

The p110 $\alpha$  subunit consist of several different domains: ABD: adapter binding domain (AKA: p85 binding domain); RBD: Ras-binding domain; C2: C2 domain; helical: helical domain; kinase: kinase domain.



### Figure 3. Structures of PI3Ks

PI3Ks are classified into three groups: class I, class II and class III. Class I PI3Ks are heterodimers consisting of a catalytic p110 subunit and a p85 regulatory subunit. The class I PI3Ks are further divided into class IA and class IB subgroups. Class IA PI3Ks consist of PI3Kα, PI3Kβ and PI3Kδ with p110α, p110β, and p110δ as their catalytic subunit respectively. The catalytic subunit can form a heterodimer with any of the p85 subunit (p85α, p85β, p55α, p50α and p55γ). The class IB PI3K subgroup consists of a p110γ catalytic subunit and one of the regulatory subunits (p101, p87 and p84). The class II and III PI3Ks consist of only a catalytic polypeptide. Several different domains are present including SH3: Src homology 3 domain; BHD: BCR homology domain; SH2: Src homology 2 domain; iSH2: Inter-Src homology 2 domain; p110γ BD: p110γ binding domain; Gβγ BD: G beta gamma protein complex binding domain; PX: phosphoinositide-binding structural domain.



**Figure 4. Protein interaction between helical domain mutation of p110α and IRS1 drives tumorigenesis**

In cancer cells harboring p110α helical domain mutations, IRS1 directly binds to mutant p110α and brings it the cell membrane, thereby activating the PI3K-AKT signaling pathway independent of any stimulation by growth factor.

**Table 1**

Mutation frequency of PIK3CA in major human cancers.

Cancer type	Mutation frequency	References
Brain	5–20%	(Samuels <i>et al.</i> , 2004, Hartmann <i>et al.</i> , 2005)
Breast	21–28%	(Bachman <i>et al.</i> , 2004, Saal <i>et al.</i> , 2005)
Cervix	36%	(Janku <i>et al.</i> , 2011)
Colorectal	30%	(Samuels <i>et al.</i> , 2004)
Endometrium	23%	(Garcia-Dios <i>et al.</i> , 2013)
Esophagus	6–11%	(Dulak <i>et al.</i> , 2013, Phillips <i>et al.</i> , 2006)
Gastric	9–25%	(Li <i>et al.</i> , 2005, Samuels <i>et al.</i> , 2004)
Liver	28–35%	(Lee <i>et al.</i> , 2005, Colombino <i>et al.</i> , 2012)
Lung	5%	(Yamamoto <i>et al.</i> , 2008)
Ovary	25%	(Levine <i>et al.</i> , 2005)
Pancreas	11%	(Schonleben <i>et al.</i> , 2006)
Thyroid	11%	(Garcia-Rostan <i>et al.</i> , 2005)